The Journal of Parasitology

Volume 28 AUGUST, 1942 Number 4

THE HELMINTHS OF RACCOONS IN EAST TEXAS

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Through the kind coöperation of Mr. Rollin H. Baker, Field Biologist of the Texas Game, Fish and Oyster Commission, 13 raccoons (*Procyon lotor lotor*) from Angelina County, in East Texas, were made available for parasitological examination. Nine of the specimens were trapped during early December; the other four were obtained in May and September. All of the raccoons were heavily parasitized, containing from four to eight species of worms. One animal was estimated to contain about 50,000 *Pharyngostomoides procyonis,* one large *Mesocestoides* and several immature ones, a dozen hookworms, a few *Molineus* and several spiny-headed worms in the intestine; eight gnathostomes in the stomach; several hundred *Eurytrema* in the pancreas; and several guinea worms in each hind foot. Two kinds of parasites, gnathostomes and guinea worms, were markedly seasonal, being abundant in winter but not at other times.

Of 12 species of helminths found, 7 are new species. Notes on each of the species encountered and descriptions of the new species follow.

Pharyngostomoides procyonis Harkema, 1942

This fluke, recently described by Harkema (1942) from raccoons in North Carolina, was found in every one of the raccoons examined. Some hosts harbored only a few dozen worms, whereas others harbored tens of thousands; one case was estimated to harbor 50,000 of these flukes.

Like many Diplostomidae this species is extremely variable in shape and size. The size is undoubtedly in part a matter of age: eggs may begin to be produced when the worms are less than 0.5 mm in length, although at this time the vitellaria are still incompletely developed. It is also possible that the number of flukes harbored and the presence of prior infections may influence the size of the worms.

Fibricola texensis Chandler, 1942

This new species of *Fibricola* is being described elsewhere, along with

Received for publication, August 5, 1941.

a complete account of its life cycle. It was found in 6 of the 13 raccoons but never in as large numbers as the *Pharyngostomoides procyonis* which always accompanied it. Its parthenitae develop in *Physa anatina* and its diplostomula in tadpoles.

Eurytrema procyonis Denton, 1942

This new species of *Eurytrema* is being described elsewhere (Denton, 1942), along with a partial account of its life cycle. It was found in the pancreas of 7 of the 13 raccoons examined, usually in large numbers. One host contained over 1000 specimens, and two others contained over 500 each. The parthenitae of this fluke develop in the land snail, *Mesodon thyroidus,* but the second intermediate host, which is probably required, has not been determined.

Mesocestoides variabilis Mueller, 1928

Adult specimens corresponding with Mueller's description of this worm were obtained from two raccoons, and immature ones, still in the tetrathyridium stage, from three others. They are discussed in more detail elsewhere (Chandler, 1942b). Some specimens obtained from a dog and from a raccoon in Nebraska, reported by Coatney (1936), have been examined by the writer, and are believed to belong to this same species. Numerous immature specimens of a *Mesocestoides* were also reported by Morgan and Waller (1940) from a raccoon in Iowa, but it was not possible to determine the species.

Since the raccoon specimens are intermediate in size between Mueller's typical *variabilis* from *Spilogale* and *Urocyon* and his variety *maior* from *Mephitis,* there no longer seems to be justification for distinguishing these varieties.

Oochoristica procyonis n. sp.

(Plate I, Figs. 1–3)

Description: Strobila 8 to 23 mm long, consisting of about 45 to 70 proglottids; maximum width 0.9 to 1.6 mm. Scolex 300 to 375 μ across and about 150 to 200 μ long, usually demarcated from neck, which is wider, by slight constriction. In contracted specimens suckers partly withdrawn into pocket-like depressions, and anterior portion of scolex retracted to give the appearance of a rostellum, as Baer (1935) figured for *O. incisa.* Suckers small, 90 to 100 μ in diameter, in dorsal and ventral pairs facing antero-dorsad and antero-ventrad; members of each pair only 23 to 32 μ apart. Neck very short, segmentation evidenced by lateral serration within 100 to 200 μ of scolex. Margins of worm serrate entire length. Segments broader than long except near end of strobila in contracted specimens, but mature segments about square and terminal segments several times as long as broad in relaxed specimens. Mature segments 0.28 to 0.7 mm long and 0.7 to 1.05 mm wide; terminal ripe segments 1 to 2.3 mm long and 0.9 to 1.6 mm broad. Genital atrium about 70 to 90 μ deep, commonly closed by a pair of lips and with a small ante-chamber into which the sperm duct opens, connected with the atrium by a minute curved duct (Fig. 1). Genital pores irregularly alternating, $\frac{1}{5}$ to $\frac{1}{7}$ length of proglottid from anterior border. Only two pairs of excretory tubes seen, the ventral ones very thin walled and about

twice diameter of dorsal ones, which pursue a wavy course; transverse tubes not seen. Genital ducts pass dorsal to excretory canals.

Testes 48 to 63, round or transversely flattened according to state of contraction of segment, measuring 30 by 40 μ to 50 by 70 μ; situated between excretory vessels, mostly posterior and lateral to female glands, a few anterior-lateral or occasionally anterior, almost as numerous on poral as aporal side, and for most part in single dorsal layer. Cirrus pouch 110 to 130 μ long and 40 to 45 μ wide, entering genital atrium indirectly through ante-chamber; inner end of cirrus pouch 200 to 220 μ from margin of proglottid, extending far beyond excretory canals. Vas deferens much coiled anterior to ovary on poral side.

Ovary large, much branched, roughly reniform, in anterior median portion of segment, occupying about half width of segment; maximum breadth in mature segments to 375 to 530 μ. Vitelline gland also consisting of clusters of lobes, tending to become somewhat confluent in older segments; maximum size 175 to 195 μ broad and 115 to 175 μ long. Vagina, opening into genital atrium from behind, nearer margin than cirrus, follows contour of cirrus for about half its length, then pursues its course to oötype in smooth curve dorsal to ovary. Seminal receptacle forms as enlargement of vagina dorsal to ovary and not far from oötype; it measures 60 to 80 μ long and 30 to 35 μ wide in mature segments, but increases to about 130 by 65 μ in post-mature segments. Mehlis' gland spherical, situated medially between ovary and vitelline gland, anterior to center of segment. Uterus rapidly spreads out over entire segment; ovary and vitelline glands quickly disappear, but testes, cirrus pouch, vagina and seminal receptacle persist almost to end. Onchospheres (in mounted segments) about 25 to 28 by 30 to 34 μ, with hooks 13 μ long.

Hosts: Procyon lotor lotor.
Location: Small intestine.
Locality: Angelina County, Texas.
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44942; Paratypes, No. 44943.

Leigh (1940) obtained 75 specimens of an *Oochoristica* from one of six raccoons in Illinois, but was unable to make a specific determination because of the immaturity of the worms. Three other species of *Oochoristica* have been reported from carnivores in the Western Hemisphere: *O. mephitis* Skinker, 1935, from *Mephitis* in Georgia; *O. oklahomensis* Peery, 1939, from *Spilogale* in Oklahoma; and *O. sandgroundi* (*Atriotaenia parva* Sandground, 1926) Baer, 1935, from *Nasua* in Brazil. *O. procyonis* differs markedly from the two skunk species in the size and shape of the cirrus pouch, and in having irregularly alternating genital pores. It most nearly resembles *O. sandgroundi* but is a larger worm, differing in having a much smaller scolex, smaller suckers, and longer cirrus pouch. Among other mammalian forms it most nearly resembles *erinacei* in most respects, but differs in having a broader scolex, smaller suckers, genital ducts dorsal to excretory canals, presence of a seminal receptacle, and more numerous testes.

Only one raccoon harbored this worm, but in this animal several dozen specimens were present.

Macracanthorhynchus ingens (v. Linst. 1879) Meyer, 1933

This acanthocephalan was found in 11 of the 13 raccoons, in numbers from 1 to about 80 per host. An account of the life cycle of this worm,

which involves a white grub as an intermediate host, is to be reported elsewhere by Donald V. Moore.

Arthrocephalus lotoris (Schwarz, 1925) n. comb.

This hookworm was found, in moderate numbers, in all of the raccoons examined. It appears to be widely distributed in raccoons in the United States as Price (1928) surmised when he found it in a civet, *Bassariscus astutus flavus* in Texas, far from Maryland, where Schwartz first found it in raccoons. In addition to the Maryland and Texas records it is almost certain that the hookworms found by Leigh (1940) in two of six raccoons in Illinois were representatives of the same species. Leigh designated his worms "Arthrocephalus sp.," and called attention to their similarity to Vaz's (1935) *A. maxillaris* except in certain characters of the buccal capsule.

Schwartz (1925) described this hookworm from a raccoon in Maryland under the name *Uncinaria lotoris*. Ortlepp (1925), earlier in the same year, erected a new genus Arthrocephalus for a new species of hookworm, *A. gambiensis,* from an African mongoose. The most striking characteristic of this genus is the presence of sutures which divide the buccal capsule into a number of articulated cuticular plates. Schwartz' *Uncinaria lotoris* possesses these sutures and has an arrangement of plates so similar to those of the type species of the genus that there can be no question that the two are cogeneric. There are, however, some characters in which these two worms do not agree, thus necessitating an emendation of the genus. Ortlepp included as characters of the genus, besides the completely articulated buccal capsule, the absence of buccal lancets, the presence of a well developed dorsal cone, and the longitudinal arrangement, without transverse coils, of the loops of the female genitalia. In none of these characters does *A. lotoris* agree with *A. gambiensis,* for the former species possesses a pair of obtuse ventral lancets, lacks a dorsal cone, and has transverse coils in the female genitalia. In all these respects it more nearly resembles members of the genus *Uncinaria,* in which Schwartz placed it, but in the writer's opinion the articulated character of the buccal capsule, shared by these two species, is sufficient reason for retaining Ortlepp's genus. The absence of ventral lancets, presence of a dorsal cone, and longitudinal arrangement of female genital organs should be dropped from the characterization of the genus.

It is possible that hookworms from *Procyon cancrivorus* in Brazil described by Vaz (1935) under the name *Arthrocephalus maxillaris* (Molin, 1860) are identical with *A. lotoris,* but Vaz's description and figures of the buccal capsule do not exactly correspond to the condition in *A. lotoris.* Vaz specifically states that no ventral lancets are present, though they are unmistakably present in *lotoris.* Although Vaz at-

tempted to identify his species with one of two briefly described by Molin in 1860 from the same host and locality, Molin's descriptions are so incomplete that, as Schwartz pointed out, they are unrecognizable. If Vaz's form from *Procyon cancrivorus* should prove to be identical with the North American form, his name *maxillaris* should be considered a synonym of *lotoris*.

Molineus barbatus n. sp.
(Plate I, Figs. 4–6)

Description: Extremely delicate red worms with finely striated cuticle and with 24 to 28 longitudinal ridges. Cephalic extremity with a coarsely striated cuticular enlargement 50–55 μ long, not greatly inflated. Diameter of head 25 to 29 μ. Annular sulcus at level of excretory pore well marked, 190 to 200 μ from anterior end. Cervical papillae not observed. Nerve ring just anterior to excretory pore. Esophagus clavate, 360 μ long in males, 420 to 480 μ long in females, with maximum diameter of 35 to 40 μ.

Females 5.5 to 6.6 mm long; maximum diameter 68 to 74 μ. Vulva salient, 0.9 to 1 mm from posterior end, dividing body about 5 : 1. Anterior ovejector about 0.9 mm long, containing 9 to 12 eggs; posterior one about 0.7 mm long containing 6 to 9 eggs. Eggs elliptical, 50 to 53 μ by 32 to 37 μ. Anus 56 to 61 μ from posterior end; tail bluntly trilobed at tip, 15 μ broad, the middle lobe bearing a very slender spine 13 to 18 μ long.

Males 4.3 to 4.7 mm long with maximum diameter of 60 to 64 μ. Bursa with large lateral lobes and medium-sized, clearly-demarcated dorsal lobe. Inner surface of lateral lobes between lateral and ventral rays studded with numerous well-defined spines, thinning out ventral to ventral rays. Ventral rays coarser than lateral, diverging only at tips, and reaching margin of bursa. Antero-lateral ray shorter, diverging from other lateral rays but remaining much nearer to them than to ventrals. Medio-lateral and postero-lateral rays contiguous, long and slender, reaching margin of bursa. Externo-dorsals thicker than any other rays, curving away from dorsal to become almost contiguous with lateral rays. Dorsal bifurcated distally, each branch again bifurcated, the inner of the terminal branches ending in two prongs, the other one single, thus giving appearance of three short prongs at end of each primary bifurcation. Spicules equal, 90 to 100 μ long, with a conspicuous medially-directed barb anteriorly, and ending in two pointed processes, outer one of which may represent two closely applied processes; inner process smaller and shorter than outer one. Gubernaculum slender, 50 to 55 μ long and only 7 μ wide.

Host: Procyon lotor lotor.
Location: Small intestine.
Locality: Angelina County, Texas.
Cotypes (♂ and ♀): U. S. Nat. Mus. Helm. Coll. No. 44930.

This species is the second of its genus to be reported from North America. Skinker, 1932, reported *M. patens,* of Old World MUSTELIDAE, from *Canis lestes* in Washington and from a mink in Mississippi. Sprehn (1932), however, described another species, *M. americanus,* from an American mink in Europe. Cameron (1936) obtained a single decomposed female *Molineus* from *Procyon cancrivorus* in Trinidad, but suspected that it belonged to the species *M. barbaris,* which he found in *Tayra barbarus* in Trinidad. The species here described is relatively more slender than any heretofore reported. It is much smaller than *M. americanus,* with spicules only half as long, and shaped differently. It

is also much smaller than *M. patens* and has much smaller and more slender spicules. The form of the spicules, arrangement of the dorsal rays, and large number of longitudinal cuticular ridges are sufficient to distinguish it from any other species so far described. This worm was obtained in small numbers from 4 of the 13 raccoons examined.

Synhimantus longigutturata n. sp.
(Plate I, Figs. 7–8)

Description: Female 3.54 mm long, with maximum diameter of 65 μ; diameter at origin of esophagus 35 μ. Two prominent lips about 8 μ long and 7 μ wide dorso-ventrally. Bottom of dorso-lateral and ventro-lateral loops of cephalic cordons 65 μ from anterior end; top of recurrent lateral loops 38 μ from anterior end. Excretory pore 230 μ from anterior end. Pharynx extending far posterior to end of cordons, to 145 μ from anterior end. Esophagus 265 μ long, in two parts; anterior glandular part 155 μ long. Vulva 1.5 mm from posterior end, dividing body about 4 : 3. Anus 96 μ from posterior end. Tail has bluntly rounded end, 9 μ in diameter. Eggs in oviducts about 40 by 24 μ. Male unknown.

Host: Procyon lotor lotor.
Location: Small intestine.
Locality: Angelina County, Texas.
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44928.

Since all known members of this and related genera of the family ACUARIIDAE are parasites of the anterior portion of the alimentary canal of birds, it is probable that the single female worm found in a raccoon had been ingested with a bird host. The finding is of interest, however, since there is only one previous record of a member of this genus in North America (*"Dispharagus egrettae* Rud. 1819" of MacCallum, 1921, = *Synhimantus invaginata* von Linst. 1901; *vide* Vigueras, 1936). The worm differs from all previously described species of the genus in its minute size and in the relatively extreme length of the buccal vestibule, which is nearly half the length of the esophagus and extends far beyond the posterior extremities of the cordon loops.

Gnathostoma procyonis n. sp.
(Plate II, Figs. 12–15)

Description: Large stout worms, tapering only slightly at the ends. Head bulb with 9 or 10 rows of spines, 90 to 100 spines in each row, set about 22 μ apart; protruding portions of hooks about 15 μ long. Body clothed with scales for approximately half its length, the scales then running together to form serrated ridges or striations around body. Posterior portion of body marked by coarse transverse wrinkles independent of the serrated striations, 50 to 100 μ apart, giving cuticle in this region a corrugated appearance. Cuticle in posterior portion of body also markedly inflated, giving body an irregular bloated appearance. Scales very large, with only three, occasionally four, principal points, but usually with one or several minor points or serrations. Immediately behind head bulb bases of scales about 20 μ broad, set about 50 μ apart, and having short blades (Fig. 13a). After first few rows bases of scales become somewhat smaller, about 15 μ broad, set about 25 to 28 μ apart, the blades becoming longer and broadening out, assuming shape of an elongated maple leaf (Figs. 13b and c). Most of scales have a long middle point and two shorter lateral points, usually with one or more minor serrations. Occa-

sionally one of minor serrations becomes large enough to make scale 4-pointed instead of 3-pointed. Throughout most of scaled area scales about 20 μ apart. Scales reach a maximum length of 80 to 85 μ with width of 20 to 25 μ. Near middle of body a rather sudden transition from rows of separate scales to serrated ridges; scales first have their points reduced to short terminal prongs, then become shortened, and finally become fused at base into continuous serrated ridges (Figs. 13e and f). Esophagus club-shaped, about one-fifth length of body. Cervical sacs 1.6 to 1.85 mm long, measured from posterior border of head bulb.

Female, 20 to 26 mm long, with a maximum diameter, where cuticle is not inflated, of about 1.1 to 1.2 mm, but where inflated in posterior portion of body may reach 1.5 to 1.6 mm. Head bulb about 700 to 800 μ broad and 350 to 370 μ long. Esophagus 4.2 to 4.3 mm long. Vulva 8.25 to 8.5 mm from posterior end, dividing body 2 : 1 or 3 : 2. Ovejector (Fig. 14), provided with three nearly spherical glands near its exit, turns forward in body, remaining narrow (240 μ in diameter) with thick muscular walls for a distance of about 2.8 mm. Here it widens out into a thin walled tube filled with eggs, 560 μ in diameter. It continues forward, narrowing to about 200 μ, and bending backward about 8 to 9 mm anterior to vulva. In some specimens it bifurcates into two posteriorly directed uteri just behind the turn, in others 2 or 3 mm behind this point. Anus about 280 μ from posterior end, which is bluntly rounded and provided with usual three papillae, the lateral ones of which, as seen in ventral view, are much broader than the median one (Fig. 12a). Extreme width across lateral papillae about 210 μ.

Male, 16 to 19 mm long, with a maximum diameter of about 800 μ where cuticle is not inflated, but expanding to 1.15 mm where inflated in posterior half of body. Head bulb 650 to 700 μ broad and 280 to 325 μ long. Esophagus 3.4 to 3.5 mm long. Anus 360 to 380 μ from posterior end, the bursa-like expansion about 850 to 900 μ in diameter. The four pairs of large lateral papillae lie close together (Fig. 15), the posterior pair slightly shorter but not more slender than the others, and not markedly separated from them. Under surface of bursa with fine spines, only slightly larger than those on posterior body striae, only on portion anterior to papillae; rest of bursa unspined. No ventral papillae seen. Left spicule short, 550 to 720 μ long, about 35 μ broad at the base, 25 μ broad in its basal half, then abruptly narrowed to about 12 μ in distal portion. Long right spicule very variable in length, 1.8 to 2.95 mm, 85 μ broad at its base, 55 μ broad in its proximal portion, narrowing to about 26 μ distally.

Host: Procyon lotor lotor.
Location: Stomach.
Locality: Angelina County, Texas.
Cotype specimens: U. S. Nat. Mus. Helm. Coll. No. 44931.

This species resembles *G. spinigerum* more closely than it does *G. didelphis.* It is smaller than *didelphis* and differs in the size and shape of the body spines; in the manner of termination of the spines, which in *procyonis,* as in *didelphis,* goes over to serrated ridges, while in *spinigerum* the spines become small but remain separate and distinct; in the inflation of the cuticle; in the size and shape of the papillae at the posterior end of the body of the female; in the shape and relative lengths of the spicules; in the lack of spination of the bursa; and in the even spacing of the lateral caudal papillae of the male. It differs from *spinigerum* in the shape of the body scales; in the transition to serrated ridges instead of separate, widely-spaced spines; in the shape of the papillae at the posterior end of the body of the female; in the lack of spination of the bursa; and in the shape and relative size of the most posterior lateral papillae of the male.

This worm was found in the stomachs of all 9 of the raccoons examined in December, in numbers from 1 to 58 per host. In one raccoon examined in May a single adult was found in the stomach, although there were scars where others had been. In 3 animals examined in September no gnathostomes were found. It seems evident that this species is of markedly seasonal occurrence. When present in numbers these are undoubtedly the most harmful parasites harbored by raccoons. The stomach wall in a few specimens was veritably riddled by them.

In the raccoon examined in May a larval female gnathostome was found encysted in the skin. It resembles those described by the writer (1925) from the liver of cats experimentally infected with cysts of *G. spinigerum* from snakes. It is 6.5 mm long and 500 μ in diameter, with the four rows of spines on the head bulb characteristic of fourth-stage gnathostome larvae. The spiny annulations of the body fade out at or slightly before the middle of the body (at the junction of the second and last thirds in *spinigerum* larvae), and the vulva is situated 72% of the body length from the anterior end (60–65% in *spinigerum* larvae). Whether this represents an aberrant larva of *G. procyonis,* or of *G. didelphis* in a foreign host, cannot be determined until larvae of both these species have been found and compared.

Dirofilaria tenuis n. sp.
(Plate I, Figs. 9–11)

Description: Females (males unknown) slender worms 8.5 to 9.6 cm long, with maximum diameter of 310 to 330 μ. Caudal end bent dorsad. Cuticle rather conspicuously marked by transverse striations about 5 μ apart, and longitudinal ridges, more or less broken and branched (Fig. 10), about 10 μ apart. Esophagus 1.12 to 1.4 mm long, and 50 μ in diameter. Nerve ring 275 to 300 μ from anterior end. Cervical papillae small, about 430 μ from anterior end. Vulva almost immediately behind end of esophagus, 1.23 to 1.5 mm from anterior end. Vagina directed posteriorly, 4.5 to 5 mm long, then dividing into two opisthodelphous uteri. Anus difficult to see, 125 to 165 μ from posterior end. Tail bluntly rounded; diameter at anus 117–130 μ. Microfilariae in blood 220 to 250 μ long and 4 to 5 μ in diameter, with finely striated cuticle. Anterior end bluntly rounded, first 9 to 10 μ without nuclei. Tail very finely drawn out. Nerve ring 20 to 24% of length from anterior end; excretory pore 35%; genital cell 60%; anal break 80%; last nucleus 90%.

Host: Procyon lotor lotor.

Location: Subcutaneous tissue of belly and flanks.

Locality: Angelina County, Texas.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44929; paratype (microfilariae) No. 44940.

This *Dirofilaria,* belonging to the skin-inhabiting division of its genus for which Faust (1937) erected the subgenus *Nochtiella,* differs from others of this group in its slenderness, the distance of the anus from the posterior end, and the cuticular markings.

Several other forms of subcutaneous *Dirofilaria* have been reported from North America. *D. scapiceps* from rabbits is a relatively short,

stout worm with short spicules. Three species (*spinosa, subcutanea,* and *repens*) were reported from porcupines by Canavan (1921, 1923), but Lent and Freitas (1937) consider these all alike; they are all larger and stouter worms than *D. tenuis.* Chitwood (1933b) examined worms from a Canadian lynx which he referred to the species *acutiuscula,* expressing the opinion, without giving reasons, that *repens* is identical with *acutiuscula,* and therefore a synonym. Some parasitologists (Faust, 1937; Lent and Freitas, 1937) have not been willing to accept Chitwood's dictum. The members of this genus are notoriously variable in size and proportions and in details of spicules and male caudal papillae, so differentiation of species is difficult. Either most of the species must be merged for lack of clear-cut morphological characters which do not overlap, or one must provisionally accept forms from different types of hosts as specifically distinct until more is known about their variations and biology. It is improbable that *acutiuscula,* found in the stomach walls of peccaries in South America, is identical with *repens,* found subcutaneously in dogs in Europe, especially when only a single case has been recorded of a subcutaneous *Dirofilaria* in South American dogs.

The cuticular markings of most species of *Dirofilaria* have not been carefully described. Fülleborn (1908) gave a figure of the cuticle of *repens* showing very fine longitudinal striations in addition to the transverse ones. Lent and Freitas (1937) stated that *D. acutiuscula* has about 70 longitudinal ridges which, according to their figure, are 16 to 24 μ apart. In *tenuis* there are about 90 ridges, conspicuously broken and branched, and spaced only about 10 μ apart. *D. immitis* shows no longitudinal ridges at all, and much less conspicuous transverse ridges. The fact that in the descriptions of other species of *Dirofilaria* the longitudinal ridges are not mentioned suggests that they must be much less conspicuous than in *tenuis.* It is possible that these markings may provide reliable specific characters in this genus.

Only one of the raccoons examined yielded specimens of this worm, and none of the others contained microfilariae in the blood. Only three female specimens were found, coiled in the subcutaneous tissue of the belly and flanks. A careful search for males was fruitless.

Dracunculus insignis (Leidy, 1858) n. comb.

A guinea worm very closely resembling the species attacking man in the Old World, *Dracunculus medinensis,* except in size, was found in 6 of the 9 raccoons examined in December, but not in any of 6 (2 not autopsied) examined in May and September. In one case living worms were found in both front and hind feet, and in one case there were dead calcified worms in a front foot and living ones in the hind feet, but in all the other cases the worms were found only in the subcutaneous tissue of one or

both hind feet, much twisted and coiled, and occupying little space for their size. The worms varied from 7.6 to 28 cm in length, with a diameter of 0.84 to 1.08 mm. Specimens with embryos were 20 to 28 cm long. In only one of the nine raccoons examined in the first half of December had an ulcer formed for the escape of the embryos. Trappers in East Texas say that such ulcers are frequently seen in January and February.

This is the first time that guinea worms have been found with regularity in any host in the United States, although a number of sporadic reports are available (Benbrook, 1932, in a fox in Iowa; Chitwood, 1933a, in raccoons in Ontario and New York and mink in Nebraska; and Benbrook, 1940, in a dog and mink in Iowa). Chitwood called attention to the probability that Leidy's (1858) *Filaria insignis* from a raccoon was also a guinea worm. Unfortunately Leidy's specimen appears now to be missing, but there seems little reasonable doubt that Leidy was describing the same worm that is here reported and that his name is acceptable for the species providing it is not identical with *D. medinensis*.

Chitwood was of the opinion that the worms from American wild carnivores are specifically identical with *Dracunculus medinensis* in spite of their much smaller size. The writer, however, is of the opinion that the cephalic structures of the North American worm do not correspond exactly with those of *D. medinensis* as described and figured by Moorthy, though they closely resemble those figured by Travassos (1934) for *D. fuelleborni* from an opossum in South America. Good specific characters are notably few in the females of this genus, and the writer, on epidemiological grounds, considers it very doubtful that *insignis* and *medinensis* are identical. It is hoped that in another season the males can be obtained from experimental infections in dogs and that these may provide definite means of identification. A fuller account of these guinea worms, with a report of a possible human case, and a discussion of the epidemiological grounds for considering them distinct from *D. medinensis,* are being published elsewhere (Chandler, 1942c).

SUMMARY

An examination of 13 raccoons from East Texas revealed the following parasites in the incidences given: *Pharyngostomoides procyonis,* 13 cases; *Fibricola texensis,* 6 cases; *Eurytrema procyonis,* 7 cases; *Mesocestoides variabilis,* adults, 2 cases, tetrathyridea, 3 cases; *Oochoristica procyonis* n. sp., 1 case; *Macracanthorhynchus ingens,* 11 cases; *Arthrocephalus lotoris,* 13 cases; *Molineus barbatus* n. sp., 5 cases; *Synhimantus longigutturata* n. sp. (probably pseudoparasitic), 1 specimen; *Gnathostoma procyonis* n. sp., 11 cases; *Dirofilaria tenuis* n. sp., 1 case; *Dracunculus insignis,* 6 cases. Each raccoon harbored from 4 to 8 species

of helminths. *Pharyngostomoides procyonis* was the most abundant parasite, *Gnathostoma procyonis* the most harmful.

Bibliography

Baer, J. G. 1927 Monographie des cestodes de la famille des Anoplocephalidae. Bull. Biol. France et Belgique Suppl. **10**: 1–241.

Benbrook, E. A. 1932 *Dracunculus medinensis* (Linnaeus, 1758) appears in the United States as a parasite of the fox. J. Am. Vet. Med. Assn. **81**: 821–824.

———1940 The occurrence of the guinea worm, *Dracunculus medinensis,* in a dog and in a mink, with a review of this parasitism. J. Am. Vet. Med. Assn. **96**: 260–263.

Cameron, T. W. M. 1936 Studies on the endoparasitic fauna of Trinidad. III. Some parasites of Trinidad carnivores. Canad. J. Research, **14**D: 25–38.

Canavan, W. P. N. 1929 Nematode parasites of vertebrates in the Philadelphia Zoological garden and vicinity. I. Parasitology **21**: 63–102.

———1931 Ibid. II. Parasitology **23**: 196–229.

Chandler, A. C. 1925 A contribution to the life history of a gnathostome. Parasitology **17**: 237–244.

———1932 Notes on the helminth parasites of the opossum. (*Didelphis virginiana*) in Southeast Texas, with descriptions of four new species. Proc. U. S. Nat. Mus. **81** (Art. 16): 1–15.

———1942a The morphology and life cycle of a new strigeid, *Fibricola texensis,* parasitic in racoons. Trans. Am. Micr. Soc. **61**: 156–167.

———1942b *Mesocestoides manteri* n. sp. from a lynx, with notes on other North American species of *Mesocestoides.* J. Parasitol. **28**: 227–231.

———1942c The guinea worm, *Dracunculus insignis* (Leidy, 1858), a common parasite of raccoons in East Texas. Am. J. Trop. Med. **22**: 153–157.

Chitwood, B. G. 1933a Does the guinea worm occur in North America? J. Am. Med. Assn. **100**: 802–804.

———1933b Note on a genus and species of nematode from *Lynx canadensis.* J. Parasitol. **20**: 63.

Coatney, G. R. 1936 Some notes on cestodes from Nebraska. J. Parasitol. **22**: 409.

Denton, Fred 1942 *Eurytrema procyonis* n. sp. (Trematoda: Dicrocoelidae) from the raccoon, *Procyon lotor.* Proc. Helm. Soc. Washington **9**: 29–30.

Dubois, G. 1938 Monographie des Strigeida (Trematoda). Mém. Soc. Neuchâteloise Sc. Nat. **6**: 1–535.

Faust, E. C. 1937 Mammalian heart worms of the genus Dirofilaria. Festsch. Nocht., pp. 131–139.

Fülleborn, F. 1908 Ueber Versuche an Hundefilarien und deren Uebertragung durch Mücken. Arch. Schiffs- u. Tropen-Hyg. **12**: 1–43.

Harkema, R. 1942 *Pharyngostomoides procyonis* n. g., n. sp. (Strigeida), a trematode from the raccoon in North Carolina and Texas. J. Parasitol. **28**: 117–122.

Hughes, R. C. 1940 The genus Oochoristica Luhe, 1898. Am. Midland Naturalist **23**: 368–381.

Leidy, J. 1858 Contributions to helminthology. Proc. Acad. Nat. Sc. Phila. **10**: 110–112.

Leigh, W. H. 1940 Preliminary studies on parasites of upland game birds and fur-bearing mammals in Illinois. Ill. Nat. Hist. Bull. **21**: 185–194.

Lent, H. and de Freitas, J. F. T. 1937 Contribução ao estudo do genero Dirofilaria Railliet and Henry, 1911. Mem. Inst. Oswaldo Cruz **32**: 37–51.

Morgan, B. B. and Waller, E. F. 1940 Severe parasitism in a raccoon (*Procyon lotor lotor* Linnaeus). Tr. Am. Micr. Soc. **59**: 523–527.

Ortlepp, R. J. 1925 On *Arthrocephalus gambiensis* n. g., n. sp., a new ankylostome from an African mongoose. J. Helm. **3**: 151–156.

Peery, H. J. 1939 A new unarmed tapeworm from the spotted skunk. J. Parasitol. **25**: 487–490.

Price, W. W. 1928 The civet, *Bassariscus astutus flavus,* a new host for *Uncinaria lotoris.* J. Parasitol. **14**: 197.

Railliet, A., and Henry, A. 1911 Sur une filaire peritoneale des porcins. Bull. Soc. Path. Exot. **4**: 386–389.

Sandground, J. H. 1926 A new mammalian cestode from Brazil. In "Medical report of the Hamilton Rice expedition to the Amazon, in conjunction with the department of tropical medicine of Harvard University, 1924–1925," Harvard Univ. Press, pp. 285–291.

Schwartz, B. 1925 A new species of hookworm from a North American raccoon. Proc. U. S. Nat. Mus. **67**, Art. 26: 1–4.

Skinker, M. S. 1932 *Molineus patens* (Dujardin, 1845) Skrjabin and Schulz, 1926, collected in the United States. J. Parasitol. **19**: 91.

————1935 A new species of *Oochoristica* from a skunk. J. Parasitol. **25**: 487–490.

Sprehn, C. 1932 Lehrbuch der Helminthologie. Berlin. 998 pp.

Travassos, L. 1934 *Dracunculus fuelleborni* n. sp., parasito de *Didelphis aurita* Wied. Mem. Inst. Oswaldo Cruz **28**: 235–237.

————1937 Revisão da familia Trichostrongylidae Leiper, 1912. Monogr. Inst. Oswaldo Cruz **1**: 1–512 pp.

Vaz, Z. 1935 Rediscrição de *Arthrocephalus maxillaris* (Molin 1860) necatorineo parasita de *Procyon cancrivorus.* Rev. Biol. e Hyg., São Paulo **6**: 9–12.

Vigueras, I. P. 1936 Notas sobre la fauna parasitologica de Cuba (Parte 1: Vermes). Mem. Soc. Cubana Hist. Nat. **10**: 53–86.

Explanation of Plates

Plate I, p. 267

Fig. 1. *Oochoristica procyonis.* Genital atrium and cirrus pouch region; note lips guarding atrium, small ante-chamber into which cirrus pouch opens, and topographic relations of male and female openings.

Fig. 2. *O. procyonis.* Scolex in partially retracted state.

Fig. 3. *O. procyonis.* Mature proglottid.

Fig. 4. *Molineus barbatus.* Anterior end.

Fig. 5. *Molineus barbatus.* Spicules and gubernaculum.

Fig. 6. *Molineus barbatus.* Posterior end of male.

Fig. 7. *Synhimantus longigutturata.* Head, lateral view; Fig. 8. Same, dorsal view.

Fig. 9. *Dirofilaria tenuis.* Anterior end of female.

Fig. 10. *Dirofilaria tenuis.* A region near middle of body showing longitudinal cuticular markings.

Fig. 11. *Dirofilaria tenuis.* Posterior end of female.

Plate II, p. 268

Fig. 12. Posterior end of females of three species of *Gnathostoma;* a, *G. procyonis;* b, *G. spinigerum;* c, *G. didelphis.*

Fig. 13. Body scales of *Gnathostoma.* Figs. a–f, *G. procyonis;* a, first row behind head bulb; b, about 1 mm behind head bulb; c, about 5 mm behind head bulb; d, about 10 mm behind head bulb; e, about 12 mm behind head bulb; f, about 12.5 mm behind head bulb. Fig. g, *G. spinigerum,* irregularly spaced spines in transition region of body, corresponding to Fig. e or f of *G. procyonis.*

Fig. 14. Vagina and beginning of uteri of *Gnathostoma procyonis.*

Fig. 15. Posterior end of small male of *G. procyonis.*

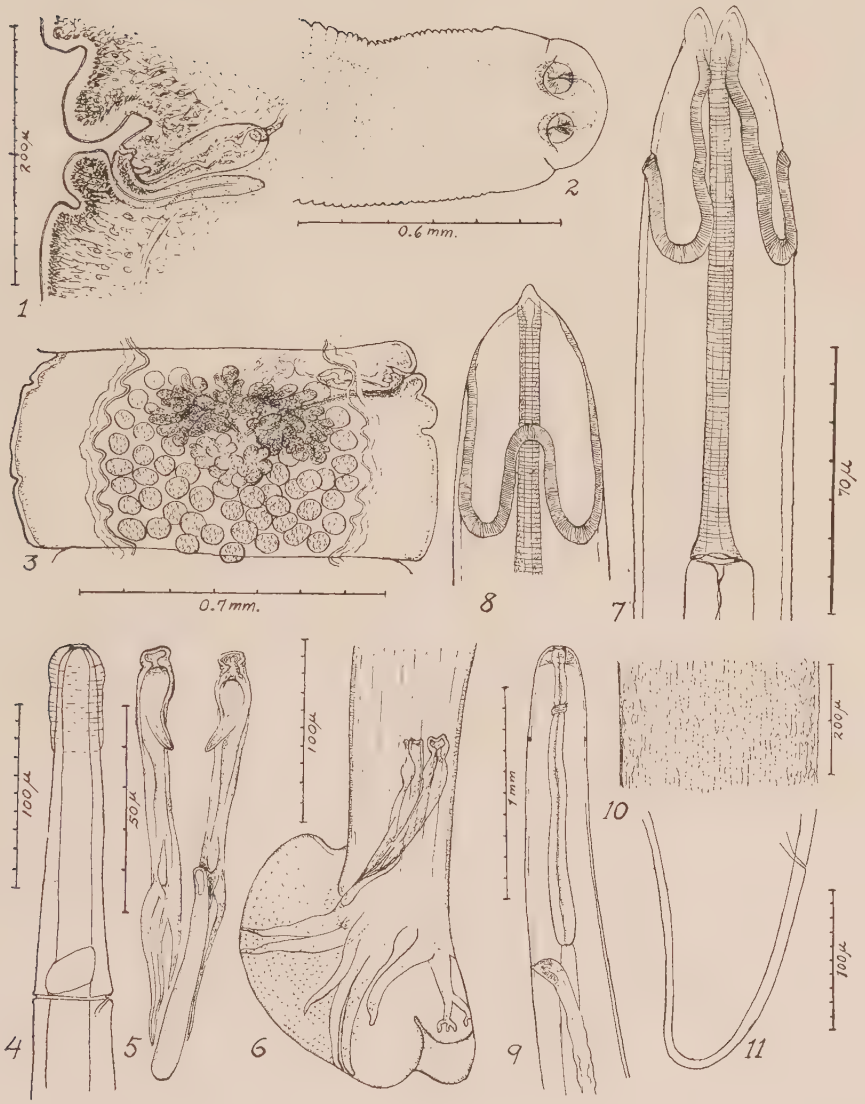
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PLATE I

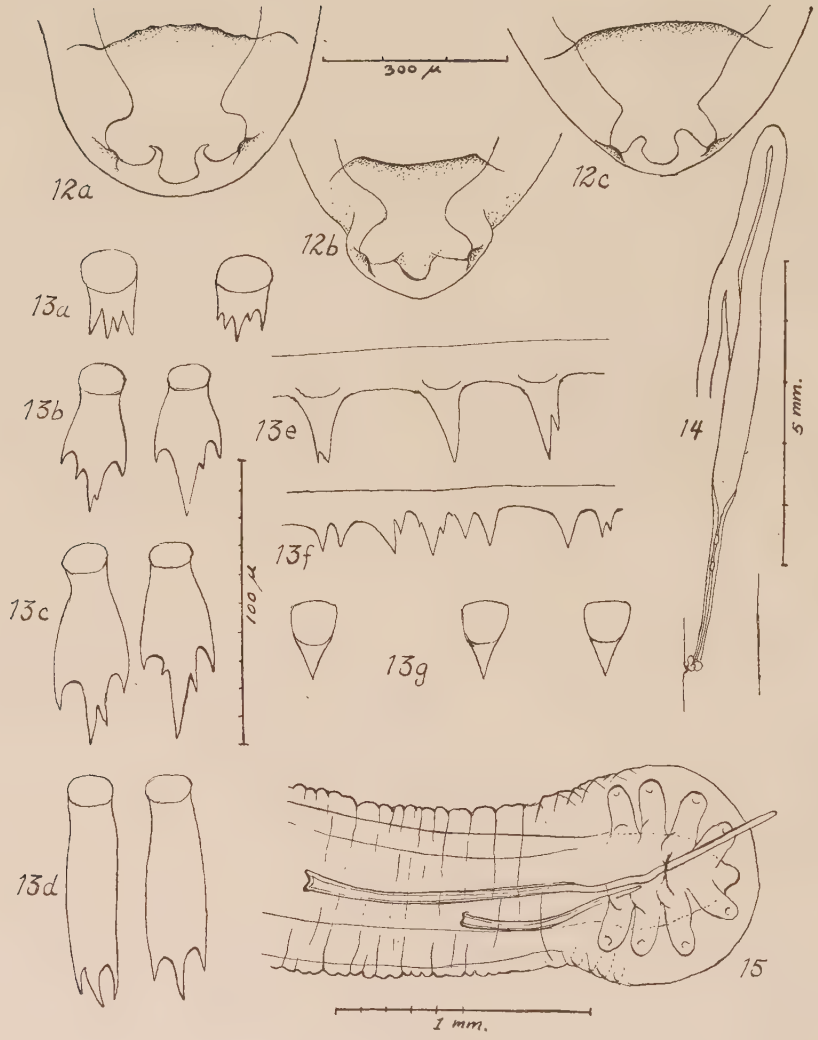

PLATE II

THREE NEW SPECIES OF *PHYLLODISTOMUM* (TREMATODA: GORGODERIDAE) FROM MICHIGAN FISHES[1]

JACOB H. FISCHTHAL

Examination of the fishes of Honey Creek near Ann Arbor, Michigan, during the spring of 1941 for trematodes of the genus *Phyllodistomum* Braun, 1899, revealed the presence of three new species occurring in the ureters and urinary bladders of the northern creek chub, *Semotilus atromaculatus atromaculatus* (Mitchill), the Mississippi Valley common shiner, *Notropis cornutus chrysocephalus* (Rafinesque), and the horny-headed chub, *Nocomis biguttatus* (Kirtland). These are described as follows:

Phyllodistomum semotili n. sp.
(Fig. 1)

Description: Phyllodistomum. Body small, spatulate, consisting of a circular, discoidal posterior portion set off at middle of acetabulum from a narrower, tapering anterior portion. Cuticula spineless, bearing sensory papillae on suckers and on surfaces and margins of body. Posterior end of body slightly notched. Oral sucker subterminal, smaller than acetabulum; diameter of oral sucker to acetabulum as 1 : 1.5. Mouth subterminal, ventral; esophagus weakly muscular, slender, narrow; intestinal crura inflated, extending nearly to posterior end of body. Vitellaria oval, smooth, near and postero-lateral to acetabulum. Vitelline ducts joined mesially; common vitelline duct short, slightly swollen.

Ovary compact, smaller than either testis, slightly and irregularly lobate, usually in contact with vitellarium, amphitypic. Oviduct short, arising from dorsum of ovary, passing antero-mesially to oötype. Mehlis' gland weakly developed, cells small. Laurer's canal arising from oviduct just before oötype and passing antero-laterally to opening on dorsal surface. Uterus fairly extensive, coils intra- and extracecal, extending to margins of body posterior to vitellaria; uterus ascending dorsal to acetabulum, constricting to weakly muscular metraterm; latter ventral to seminal vesicle; genital atrium small. Eggs fairly numerous; older intrauterine eggs containing fully developed miracidia; latter possessing two flame cells; hatching occurring as soon as eggs enter water.

Testes oblique, fairly close together, irregularly lobate, indentations deeper and lobes more numerous than in ovary. Anterior testis usually in contact with vitellarium and opposite or slightly oblique to ovary. Posterior testis directly behind but never in contact with ovary. Vasa efferentia arising from dorsum of testes, passing forward dorsal to acetabulum to their junction slightly anterior to acetabulum; vas deferens short; seminal vesicle large; ejaculatory duct short. Genital pore mid-ventral, immediately posterior to intestinal bifurcation. Excretory pore postero-dorsal; bladder a slender, median tube extending anteriorly to level of ovary where right and left primary collecting ducts enter; latter extending anteriorly to intestinal bifurcation, then looping posteriorly a short distance each receiving two secondary collecting ducts.

Average measurements in millimeters (with maximum lengths and widths) of

Received for publication, September 8, 1941.

[1] Contribution from the Department of Zoölogy, University of Michigan.

10 specimens fixed in Bouin's fluid, stained in Mayer's paracarmine and mounted in clarite X:[2] Body, length 1.481 (1.275–1.620), width at intestinal bifurcation 0.268 (0.195–0.30), at anterior testis 0.608 (0.495–0.735); oral sucker, diameter 0.188 (0.150–0.203); acetabulum, diameter 0.284 (0.225–0.30); esophagus, length 0.148 (0.120–0.195); left vitellarium, length (short axis) 0.065 (0.059–0.072), width (long axis) 0.124 (0.095–0.152); right vitellarium, 0.063 (0.053–0.075) × 0.130 (0.107–0.150); ovary, 0.139 (0.120–0.158) × 0.154 (0.130–0.195); older embryonated intrauterine eggs, 0.040 (0.037–0.043) × 0.027 (0.024–0.028); anterior testis, 0.246 (0.225–0.285) × 0.205 (0.150–0.255); posterior testis, 0.250 (0.225–0.285) × 0.218 (0.150–0.270).

Host: *Semotilus atromaculatus atromaculatus* (Mitchill).
Habitats: Ureters and urinary bladder.
Locality: Honey Creek near Ann Arbor, Washtenaw County, Michigan.
Types: U. S. Nat. Mus. Helm. Coll. No. 44947 (3 slides, type and 2 paratypes); other paratypes in author's collection.

Of 43 northern creek chubs, 8 (approximately 18.6 per cent) were infected with mature *P. semotili.* As many as 60 adult worms have been recovered from the ureters and urinary bladder of a single host.

Of 57 *P. semotili* examined, 24 had the ovary on the left and 33 had it on the right, indicating that amphitypy occurs frequently in this species.

Comparison with other species of the genus: To date 39 species of *Phyllodistomum* have been described. It is not within the scope of this paper to discuss the validity of these species, since Nybelin (1926), Holl (1929), Lewis (1935), Wu (1938) and others have already done so in their papers. The comparisons of *P. semotili* and the two new species following with the species described through 1930 are based largely upon data tabulated by Lewis (1935); all comparative data beyond his work are based upon information from the original descriptions.

Smooth vitellaria differentiate *semotili* from *acceptum* Looss, 1901, *americanum* Osborn, 1903, *angulatum* v. Linstow, 1907, *brevicecum* Steen, 1938, *carolini* Holl, 1929, *caudatum* Steelman, 1938, *conostomum* (Olsson, 1876), *elongatum* Nybelin, 1926, *entercolpium* Holl, 1930, *fausti* Pearse, 1924, *folium* (v. Olfers, 1816), *hunteri* (Arnold, 1934), *lacustri* (Loewen, 1929), *linguale* Odhner, 1902, *lysteri* Miller, 1940, *mogurndae* Yamaguti, 1934, *parasiluri* Yamaguti, 1934, *patellare* (Sturges, 1897), *pearsei* Holl, 1929, *pseudofolium* Nybelin, 1926, *sinense* Wu, 1937, *singulare* Lynch, 1936, *spatula* Odhner, 1902, *spatulaeforme* Odhner, 1902, *staffordi* Pearse, 1924, *superbum* Stafford, 1904 and *undulans* Steen, 1938.

It differs from *lesteri* Wu, 1938, *shandrai* Bhalerao, 1937 and *unicum* Odhner, 1902 in having a slightly and irregularly lobate ovary, and from *marinum* Layman, 1930, *solidum* Rankin, 1937 and *spatula* in having both ovary and testes irregularly lobate. An oral sucker smaller than the acetabulum distinguishes it further from *brevicecum, caudatum, mogurndae, pearsei* and *unicum.*

The extension of extrauterine coils as far forward as the vitellaria

[2] Clarite X was originally described as Nevillite no. 1 by Groat (1939).

and laterally to the extreme margins distinguishes it from *almorii* Pande, 1937 and *macrobrachicola* Yamaguti, 1934, and also from *americanum, angulatum, caudatum, conostomum, elongatum, folium, lacustri, lesteri, linguale, lysteri, marinum, pseudofolium, spatula, spatulaeforme, staffordi* and *unicum.*

The presence of a slight posterior notch differentiates it from *kajika* (Ozaki, 1926) and *lohrenzi* (Loewen, 1935), and also from *acceptum, almorii, brevicecum, carolini, caudatum, conostomum, entercolpium, fausti, hunteri, lacustri, lesteri, linguale, parasiluri, patellare, pearsei, shandrai, sinense, singulare, solidum, staffordi, undulans* and *unicum.*

Vitellaria that are from one-half to four-fifths the size of the ovary distinguish it from *megalorchis* Nybelin, 1926, and *simile* Nybelin, 1926, and also from *acceptum, americanum, conostomum, shandrai, spatula* and *superbum.* It differs from *macrocotyle* (Lühe, 1909) in that the testes and eggs are significantly smaller.

A significantly smaller ovary distinguishes it from all other species with the exception of *angulatum, brevicecum, carolini, elongatum, hunteri, macrobrachicola, marinum, pearsei* and *pseudofolium.* An ovary significantly smaller than either testis differentiates it further from *brevicecum, macrobrachicola, mogurndae, pearsei, solidum, undulans* and *unicum.*

Its significantly smaller body differentiates it from all other species with the exception of *carolini, elongatum, lacustri* and *pearsei.* Its host, a fish, distinguishes it further from *almorii, americanum, entercolpium, kajika, lesteri, patellare, shandrai, singulare* and *solidum.*

P. semotili most closely resembles *almorii, carolini, kajika, megalorchis, parasiluri, simile, sinense* and *staffordi* from which it has already been distinguished.

Phyllodistomum notropidus n. sp.
(Fig. 2)

Description: Phyllodistomum. Body small, spatulate, consisting of a rounded, discoidal posterior portion somewhat set off at middle of acetabulum from a narrower, tapering anterior portion. Cuticula spineless, bearing sensory papillae on suckers and on surfaces and margins of body. Posterior end of body slightly notched. Oral sucker subterminal, smaller than acetabulum; diameter of oral sucker to acetabulum as 1 : 1.37. Mouth subterminal, ventral; esophagus weakly muscular, slender, narrow; intestinal crura inflated, extending nearly to posterior end of body. Vitellaria oval, smooth or slightly lobate, very close to acetabulum, occasionally overlapping it. Vitelline ducts joined mesially; common vitelline duct short, slightly swollen.

Ovary compact, equal to either testis or larger, slightly and irregularly lobate, usually in contact with vitellarium, amphitypic. Oviduct short, arising from dorsum of ovary, passing antero-mesially to oötype. Mehlis' gland weakly developed, cells small. Laurer's canal arising from oviduct just before oötype and passing antero-laterally to opening on dorsal surface. Uterus fairly extensive, coils intra- and extracecal, extending to margins of body posterior to acetabulum; uterus ascending dorsal to acetabulum, constricting to weakly muscular metraterm; latter ventral to seminal vesicle; genital atrium small. Eggs fairly numerous; older intrauterine eggs containing fully developed miracidia; hatching occurring as soon as eggs enter water.

Testes oblique, fairly close together, irregularly lobate, indentations deeper and lobes more numerous than in ovary. Anterior testis directly behind but not in contact with vitellarium, opposite or very slightly oblique to ovary. Posterior testis directly behind but never in contact with ovary. Vasa efferentia arising from dorsum of testes, passing forward dorsal to acetabulum to their junction slightly anterior to acetabulum; vas deferens short; seminal vesicle large; ejaculatory duct short. Genital pore mid-ventral, posterior to intestinal bifurcation. Excretory pore posterodorsal; bladder a slender, median tube extending anteriorly to level of ovary where right and left primary collecting ducts enter; latter extending anteriorly to intestinal bifurcation, then looping posteriorly a short distance each receiving two secondary collecting ducts.

Average measurements in millimeters (with maximum lengths and widths) of 8 specimens fixed in Bouin's fluid, stained in Mayer's paracarmine or Delafield's haematoxylin and mounted in clarite X: Body, length 1.351 (0.998–1.875), width at intestinal bifurcation 0.232 (0.195–0.285), at anterior testis 0.488 (0.405–0.645); oral sucker, diameter 0.177 (0.155–0.210); acetabulum, diameter 0.243 (0.210–0.308); esophagus, length 0.137 (0.109–0.176); left vitellarium, length 0.094 (0.056–0.133), width 0.099 (0.055–0.120); right vitellarium, 0.096 (0.067–0.133) × 0.097 (0.069–0.112); ovary, 0.141 (0.104–0.180) × 0.153 (0.117–0.210); older embryonated intrauterine eggs, 0.041 (0.038–0.043) × 0.026 (0.024–0.028); anterior testis, 0.126 (0.069–0.184) × 0.104 (0.070–0.128); posterior testis, 0.143 (0.117–0.198) × 0.112 (0.080–0.165).

Host: Notropis cornutus chrysocephalus (Rafinesque).

Habitats: Ureters and urinary bladder.

Locality: Honey Creek near Ann Arbor, Washtenaw County, Michigan.

Types: U. S. Nat. Mus. Helm. Coll. No. 44949 (3 slides, type and 2 paratypes); other paratypes in author's collection.

Of 136 Mississippi Valley common shiners, 9 (approximately 6.6 per cent) were infected with mature *P. notropidus*. As many as 17 adult worms have been recovered from the ureters and urinary bladder of a single host.

Of 13 *P. notropidus* examined, 8 had the ovary on the left and 5 had it on the right, indicating that amphitypy is of frequent occurrence in this species.

Comparison with other species of the genus: An ovary equal to either testis or larger decidedly distinguishes *notropidus* from *acceptum, almorii, americanum, angulatum, carolini, caudatum, conostomum, elongatum, entercolpium, fausti, folium, hunteri, kajika, lacustri, lesteri, linguale, lohrenzi, lysteri, macrocotyle, marinum, megalorchis, parasiluri, patellare, pseudofolium, semotili, shandrai, simile, sinense, singulare, spatula, spatulaeforme, staffordi* and *superbum*.

It differs from *brevicecum, mogurndae, pearsei* and *unicum*, and further from *caudatum* in that the oral sucker is smaller than the acetabulum. It differs from *solidum*, and further from *lesteri, marinum, shandrai, spatula* and *unicum* in having an irregularly lobate ovary.

The extension of extrauterine coils as far forward as the acetabulum and laterally to the extreme margins distinguishes it from *macrobrachicola*, and also from *almorii, americanum, angulatum, caudatum, conostomum, elongatum, folium, lacustri, lesteri, linguale, lysteri, marinum, pseudofolium, spatula, spatulaeforme, staffordi* and *unicum*.

It differs further from *macrobrachicola* in that the body is somewhat smaller, the ovary slightly larger and the eggs significantly smaller. It has a body and ovary significantly smaller, but its eggs are significantly larger than in *undulans*.

The presence of a slight posterior notch further differentiates it from *acceptum, almorii, brevicecum, carolini, caudatum, conostomum, entercolpium, fausti, hunteri, kajika, lacustri, lesteri, linguale, lohrenzi, parasiluri, patellare, pearsei, shandrai, sinense, singulare, solidum, staffordi, undulans* and *unicum*. Its host, a fish, distinguishes it further from *almorii, americanum, entercolpium, kajika, lesteri, patellare, shandrai, singulare* and *solidum*.

P. notropidus most closely resembles *macrobrachicola, mogurndae, pearsei, semotili* and *solidum* from which it has already been distinguished.

Phyllodistomum nocomis n. sp.
(Fig. 3)

Description: Phyllodistomum. Body small, lanceolate, not sharply set off into narrow anterior and discoidal posterior portions. Cuticula spineless, bearing sensory papillae on suckers and on surfaces and margins of body. Posterior end of body usually slightly notched. Oral sucker subterminal, smaller than acetabulum; diameter of oral sucker to acetabulum as 1 : 1.39. Mouth subterminal, ventral; esophagus weakly muscular, slender, narrow; intestinal crura inflated, extending nearly to posterior end of body. Vitellaria oval, smooth, near and postero-lateral to acetabulum, occasionally overlapping it. Vitelline ducts joined mesially; common vitelline duct short, slightly swollen.

Ovary compact, very small, slightly and irregularly lobate, usually in contact with vitellarium, amphitypic. Oviduct short, arising from dorsum of ovary, passing antero-mesially to oötype. Mehlis' gland weakly developed, cells small. Laurer's canal arising from oviduct just before oötype and passing antero-laterally to opening on dorsal surface. Uterus sparse, coils mostly intracecal, extracecal coils few, sometimes extending to ovary and to margins of body; uterus ascending dorsal to acetabulum, constricting to weakly muscular metraterm; latter ventral to seminal vesicle; genital atrium small. Eggs few; older intrauterine eggs containing fully developed miracidia; hatching occurring as soon as eggs enter water.

Testes oblique, fairly close together, irregularly lobate, indentations deeper and lobes more numerous than in ovary. Anterior testis directly behind but not in contact with vitellarium; opposite or slightly oblique to ovary. Posterior testis directly behind but never in contact with ovary. Vasa efferentia arising from dorsum of testes, passing forward dorsal to acetabulum to their junction slightly anterior to acetabulum; vas deferens short; seminal vesicle very large; ejaculatory duct short. Genital pore mid-ventral, posterior to intestinal bifurcation. Excretory pore postero-dorsal; bladder a slender, median tube extending anteriorly to level of ovary where right and left primary collecting ducts enter; latter extending anteriorly to intestinal bifurcation, then looping posteriorly a short distance each receiving two secondary collecting ducts.

Average measurements in millimeters (with maximum lengths and widths) of 10 specimens fixed in Bouin's fluid, stained in Ehrlich's acid haematoxylin and mounted in clarite X: Body, length 1.016 (0.965–1.058), width at intestinal bifurcation 0.199 (0.180–0.218), at anterior testis 0.330 (0.308–0.345); oral sucker, diameter 0.140 (0.135–0.143); acetabulum, diameter 0.195 (0.180–0.205); esophagus, length 0.118 (0.093–0.135); left vitellarium, length 0.046 (0.037–0.061), width 0.069 (0.045–0.077); right vitellarium, 0.050 (0.040–0.064) × 0.073 (0.053–0.093); ovary, 0.066 (0.059–0.080) × 0.076 (0.059–0.096); older embryonated intrauterine eggs, 0.040

(0.037–0.045) × 0.026 (0.024–0.027); anterior testis, 0.103 (0.093–0.120) × 0.087 (0.053–0.109); posterior testis, 0.129 (0.120–0.139) × 0.096 (0.061–0.113).

Host: Nocomis biguttatus (Kirtland).

Habitats: Ureters and urinary bladder.

Locality: Honey Creek near Ann Arbor, Washtenaw County, Michigan.

Types: U. S. Nat. Mus. Helm. Coll. No. 44948 (3 slides, type and 2 paratypes); other paratypes in author's collection.

Of 2 horny-headed chubs, 1 was infected with 31 mature *P. nocomis.* The adult worms were recovered from the ureters and urinary bladder.

Of 28 *P. nocomis* examined, 10 had the ovary on the left and 18 had it on the right, indicating that amphitypy is of frequent occurrence in this species.

Comparison with other species of the genus: A significantly smaller ovary distinguishes *nocomis* from all other species of *Phyllodistomum.* Smooth vitellaria differentiate it further from *acceptum, americanum, angulatum, brevicecum, carolini, caudatum, conostomum, elongatum, entercolpium, fausti, folium, hunteri, lacustri, linguale, lysteri, mogurndae, parasiluri, patellare, pearsei, pseudofolium, sinense, singulare, spatula, spatulaeforme, staffordi, superbum* and *undulans.*

A slightly and irregularly lobate ovary differentiates it further from *lesteri, marinum, shandrai, solidum, spatula* and *unicum.* It differs from *brevicecum, caudatum, mogurndae, pearsei* and *unicum* in that the oral sucker is smaller than the acetabulum. With the exception of *angulatum, elongatum, lacustri, lesteri, linguale, macrobrachicola* and *staffordi* a sparsely coiled uterus distinguishes it from all other species, including *almorii, kajika, lohrenzi, megalorchis, notropidus, semotili* and *simile.*

A significantly smaller body and eggs, and vitellaria that are only slightly smaller than the ovary differentiate it further from *macrobrachicola.* It differs further from *macrocotyle* in having a significantly smaller body, testes and eggs. Its host, a fish, distinguishes it further from *almorii, americanum, entercolpium, kajika, lesteri, patellare, shandrai, singulare* and *solidum.*

P. nocomis most closely resembles *almorii, angulatum, elongatum, kajika, lesteri, linguale, macrobrachicola, notropidus* and *semotili* from which it has already been distinguished.

Bibliography

Groat, R. A. 1939 Two new mounting media superior to Canada balsam and gum damar. Anat. Rec. **74**: 1–6.

Holl, Fred J. 1929 The phyllodistomes of North America. Tr. Am. Micr. Soc. **48**: 48–53.

Lewis, F. J. 1935 The trematode genus *Phyllodistomum* Braun. Tr. Am. Micr. Soc. **54**: 103–117.

Nybelin, O. 1926 Zur Helminthenfauna der Süsswasserfische Schwedens I. Phyllodistomen. Göteborgs K. Vetensk.—o. Vitterhets—Samh. Handl. 4th Ser. **31**: 1–29.

Wu, Kuang 1938 Progenesis of *Phyllodistomum lesteri* sp. nov. (Trematoda: Gorgoderidae) in freshwater shrimps. Parasitology **30**: 4–19.

PLATE I

All figures drawn to scale with the aid of a microprojector; value of scale is 0.2 mm.

FIG. 1. *Phyllodistomum semotili,* adult, ventral view.
FIG. 2. *Phyllodistomum notropidus,* adult, ventral view.
FIG. 3. *Phyllodistomum nocomis,* adult, ventral view.

THE LIFE HISTORY OF *POROCEPHALUS CROTALI*, A PARASITE OF THE LOUISIANA MUSKRAT[1]

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A preliminary report of the incidence of nymphal porocephaliasis of the Louisiana muskrat has already been published (Penn and Martin, 1941). The present paper is to report what is known of the life history of *Porocephalus crotali* in Louisiana as a result of studies in the past year, and to add a few additional data on the incidence and epidemiology.[2]

HOSTS AND DISTRIBUTION

Porocephalus crotali was first described by Humboldt in 1808 as *Echinorhynchus crotali.* Other important synonyms for the adult are *Pentastoma proboscideum* Rudolphi, *Porocephalus humboldtii* Mayer, *Linguatula quadriuncinata* Mayer and *Pentastoma moniliforme* Megnin; and, for the nymphal form, *Pentastoma subcylindricum* Diesing.

Porocephalus crotali, according to Sambon (1922), has a limited distribution, the adults being found principally in New World crotaline snakes, especially the species of *Crotalus* which "range from Arizona, New Mexico and Texas to southern Brazil and northern Argentina." The type specimen was taken from the South American rattlesnake, *Crotalus durissus terrificus* Laurenti. It has also been recorded from *C. durissus durissus, C. atrox, C. horridus* ssp., and *C. adamanteus.* Dr. Howard R. Hill at the Los Angeles Museum states[3] that he has found *P. crotali* commonly in the water-moccasin (*Agkistrodon piscivorus*); this reptile has also been recorded as a host of the adult by the writer (1940).

Sambon (1922) and Heymons (1935) state that the nymphal form has been reported from several Brazilian mammals (MARSUPIALIA: the

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Received for publication, September 8, 1941.

[1] A contribution from the Department of Zoology, the Tulane University of Louisiana, New Orleans.

[2] It is a pleasure to acknowledge the cooperation of Dr. Carl E. Venard, Ohio State University, for identification of the pentastomes; of Florence Brooks Kreeger and Dr. Harley N. Gould, Tulane University, in securing some of the muskrats; of Harry E. Adams, Julian A. Howard and the rest of the personnel of the Sabine National Wildlife Refuge, Louisiana, in supplying muskrats; and of Ruffin T. Perkins, Jr., in securing live water-moccasins and other experimental animals. Dr. F. H. Wilson, Tulane University, read and criticized the manuscript and offered a number of valuable suggestions.

[3] In a personal communication, September 24, 1940.

murine opossum *Marmosa murina* L. and *Caluromys philander* L.; CHIROPTERA: *Phyllostoma discolor* Wagn.; EDENTATA: *Tatusia novemcincta* L.; CARNIVORA: *Procyon cancrivorus* Cuv. and *Pteronura brasiliensis* Zim.; SIMIAE: *Mystax chrysopygus* Wagn.; and RODENTIA: *Rhipidomys pyrrhorhinus* Wied and *Akodon fuliginosus* Wagn.), but that the determination of the parasites has been uncertain in the majority of the cases. The nymph was first recorded from the viscera of the Louisiana muskrat (*Ondatra zibethica rivalica*) by the writer (1940).

THE LIFE HISTORY

Adults live in the lung cavities of the reptilian host. The eggs, which reach the outside in sputum, are ingested by the muskrat and hatch in the small intestine. The larvae migrate to the liver and lungs of the muskrat where they become encapsulated in the tissues, and develop into nymphs in about three months. The tissue with infective nymphs is then ingested by the reptile host and the nymphs migrate up the esophagus and into the tracheae and lungs where they develop into adults.

THE EGGS

Description.—The eggs, Fig. 1, are fully developed when deposited by the female porocephalid inside the pleural cavity of the snake host. The fully formed larva is enclosed in an inner oval-shaped vitelline membrane which in 12 eggs averaged 123 microns long (from 114 to 128) and 105 microns wide (99 to 106). The outer membranous shell is much larger, perfectly spherical in shape, and has a wide clear area between it and the inner membrane; its diameter averaged 151 microns in 12 eggs (from 135 to 170).

Productivity.—The number of eggs contained in the uterus of a 64 mm female porocephalus was calculated to be approximately 1,088,000. This must be considered as only a rough estimate as it was made by measuring the length of the uterus containing eggs and cutting out pieces as nearly as possible one millimeter long. The sections were then measured exactly and the eggs squeezed out on slides and counted exactly. Because the uterus is the same diameter throughout its length, and the eggs are nearly the same size regardless of the stage of development, the average of only four such counts (which showed little variation) was used in the final calculation. The uterus of this female was 918 mm long and the count per 0.1 mm averaged 118 eggs (100 to 150).

Viability.—On August 18, 1940 a gravid female *P. crotali* just removed from a water-moccasin was cut into shreds and placed in about eight ounces of marsh-lagoon water diluted with well-water to a salinity of approximately 0.006% (equal to 0.32% sea water). From time to time as the worm decayed the suspension of eggs was examined micro-

scopically and all of the embryonated eggs found viable. The last so examined, 189 days after having been removed from the living parent, hatched into motile larvae on slides under observation.

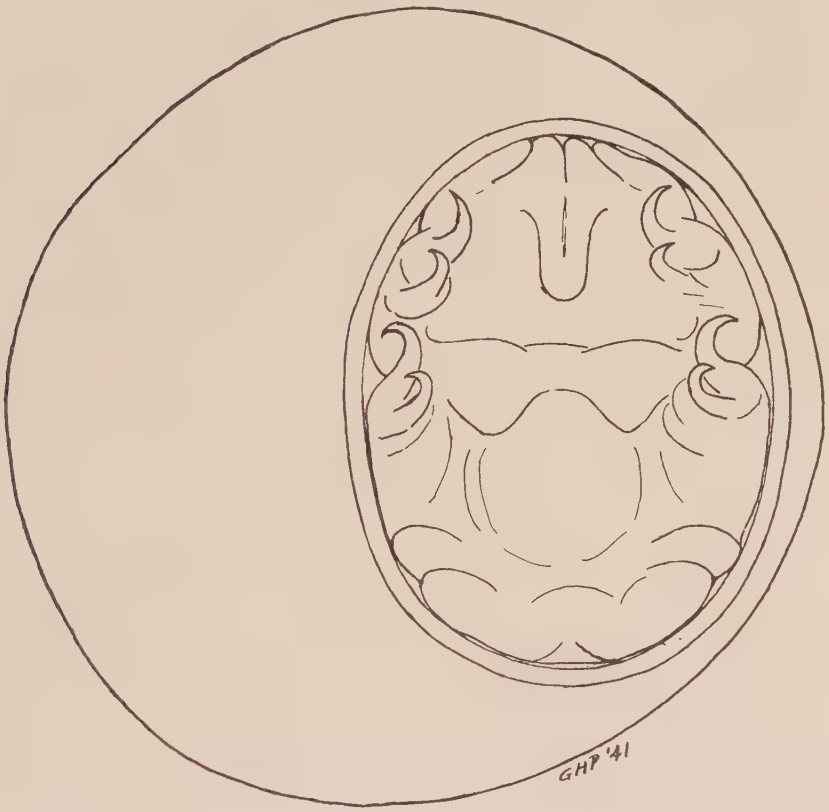

FIG. 1. *Porocephalus crotali:* embryonated egg.

THE LARVAE

Hatching.—The eggs hatch by two stages (as observed when they were placed on slides under cover glasses). First, the inner vitelline membrane splits into halves from the head end, and the larva, Fig. 2, stretches to its full size of approximately 141 microns long and 64 microns wide, while still inside the outer shell. After an indefinite period of more or less constant action by the larva, the outer shell is split into halves in much the same fashion as was the inner shell, and the larva emerges. By continued slow, strong strokes with its four, doubly hooked legs the larva frees itself of the egg cases and progresses on its migration to the site of encapsulation.

Migration of the Larvae.—From several experiments in which laboratory white rats were killed at a series of short intervals after being

orally infected with porocephalid eggs, the place of hatching was determined to be the duodenum. Both embryonated and non-embryonated eggs were used so that the level reached by the eggs was always marked by the presence of non-embryonated eggs at least. One hour after feeding, both types of eggs were recovered from the stomach, but none in the intestine. After two and four hours respectively non-embryonated eggs were recovered in the duodenum and jejunum but none of the others. Evidently all embryonated eggs hatched in the duodenum.

FIG. 2. *Porocephalus crotali:* larva, just hatched; ventral side.

No larvae were recovered from any of the rats, although each examination included the lumen of the stomach, duodenum, jejunum, the peritoneum of the stomach, duodenum, jejunum and surfaces of the liver, therefore leaving the route of migration still a matter of conjecture. Because of the size of the larvae they cannot pass through the blood capillaries of the hepatic-portal system, and a blood route therefore seems out of the question. The most probable route seems to be either through the bile ducts from the duodenum to the hepatic tissue and from there to the surface of the liver, or through the intestinal wall directly into the abdominal cavity and then to the surfaces of the liver and other viscera.

The latter, although unsupported by experimental data, appears the more favorable to the writer especially when the relatively larger number of nymphs which encapsulate in the omenta of experimental white rats is considered. A direct route through the wall of the duodenum to the omenta seems indicated.

THE NYMPHS

When the larva reaches the end of its migration, which is usually in the liver, it continues its post-larval development. It becomes imbedded in the tissue in which it has settled and shortly afterward is encapsulated by host-tissue reaction. Within the capsule the larva develops into a worm-like nymph. The nymph grows and enlarges the capsule until it is fully developed in about 100 days.

Experimental.—White rats forced to ingest viable eggs on August 27th were heavily infected with nearly developed nymphs 93 days later on November 29th. These nymphs were smaller than those obtained from muskrats, and were probably not quite fully developed; nevertheless, some of these lived in the experimental reptile host, two out of three baby water-moccasins being successfully infected with 15 mm immature porocephalids after 35 days. On the other hand, all of the encapsulated nymphs (of unknown ages) obtained from muskrats on the same date were infective and developed into post-nymphal forms when fed to water moccasins.

The fully developed nymph makes a capsule of 5.0 mm (from 4.5 to 6.0) diameter, usually in the liver of the muskrat, but also possibly in or on every organ of the visceral and pleural cavities. Only one or two nymphs are normally present in a naturally infected muskrat, where they are almost invariably located at the periphery of the hepatic tissue; the lungs, omentum and gonads, however, also are frequently parasitized, and in that order of incidence.

The nymphs themselves when removed from the capsules averaged 12.2 mm long (from 11.0 to 13.0) in the specimens measured. The number of annulations varied between 38 and 42, averaging about 40, on twenty nymphs obtained from naturally infected muskrats.

The possibility that additional hosts of the nymphal form exist in Louisiana has not been thoroughly investigated as yet, although three *Rattus norvegicus* and nine *Mus musculus* from the vicinity of the Sabine Refuge headquarters examined by the writer were found negative. Other small marsh rodents of the same locality also should be examined for the nymphal form of this porocephalus.

THE ADULTS

In laboratory-raised, baby water-moccasins four months old and in wild water-moccasins collected at Westwego, La., not over six months old

(26.0 to 27.0 cm long) it was possible to culture post-nymphal or immature-adult *Porocephalus crotali.*

Among these experimental water-moccasins four were forcibly fed with encapsulated nymphs obtained fresh from muskrats trapped at Abbeville, La., November 29th. All of the snakes proved to be satisfactory hosts for the development of the post-nymphal stages. However, one of the snakes was found dead on January 3rd with two 20 mm immature porocephalids clogged in its trachea, suffocation probably being the cause of death. Three water-moccasins of the same lot were infected orally with encapsulated nymphs from experimental white rats as described in the preceding section.

Twenty-five water-moccasins (*Agkistrodon piscivorus*) collected near the headquarters of the Sabine Refuge in Cameron Parish, La., were autopsied between August 12th and 19th, 1940. Seven were infected with adult and immature porocephalids, a higher percentage of females than males being infected. A few pentastomes of the species *Kiricephalus coarctatus* also were obtained from these moccasins. The range of the moccasins was from 39 to 120 cm, 17 were males, 7 females and one juvenile. Three additional water-moccasins from the Chalmette and Pearl River swamps in eastern Louisiana proved to be negative for *Porocephalus.* Twelve alligators ranging from 18 inches to five feet in length and a wild hog from the Sabine Refuge also were examined for adult porocephaliasis, but found negative.

In the laboratory various cold-blooded vertebrates were forcibly infected per os with encapsulated nymphs freshly obtained from wild muskrats. The experimental animals were examined twelve days after infection. Of three water snakes (*Natrix sipedon confluens*) two became infected; of three ribbon snakes (*Thamnophis sauritus proximus*) two became infected; one mobilian turtle (*Pseudemys scripta troosti*) became infected; of two small green frogs (*Rana clamitans*) one became infected. One mud-turtle (*Sternotherus odoratus*), one baby alligator (*Alligator mississippiensis*) and one tree frog (*Hyla cinerea cinerea*) failed to become infected.

OCCURRENCE OF THE NYMPHS IN MUSKRATS

Preliminary notes of porocephaliasis in Louisiana muskrats by Penn and Martin (1941) gave the figure of 9.06% as the incidence for the state of Louisiana in the 1939–40 trapping season. Of an additional 748 muskrats examined in the 1940–41 trapping season, 11.5% were found with porocephaliasis.

Data of the sexes, size and degree of infection of the muskrats were collected in the latter survey. Only a slight difference between sexes is shown, the females having a slightly higher percentage of infection than

the males. It is interesting to note also that only the larger, i.e., sexually mature (25 to 33 cm) muskrats were infected although many smaller ones were examined from the same areas.

Geographic difference in intensity of infection in 1940–41 similar to that reported in the previous study again indicates that porocephaliasis is dependent on the relative numbers of hosts of the parasite living in any particular marsh. It is obvious that the heavier the concentration of muskrats or of water-moccasins, or both on any area, the greater will be the chance for infection to increase in both hosts. Concentrations may be caused by natural fecundity of the muskrats coupled with abundance of native food-grasses and water, or may occur in drought years when muskrats congregate in the vicinity of small pools of fresh water. In either instance, the water-moccasins will be found seeking their prey and at the same time distributing the eggs of the parasite. In regard to moccasins in marshes, Arthur (1931) wrote that it "has been an old adage in the Louisiana marshes that wherever the poisonous moccasin snake is plentiful one is certain to find many muskrats. On the other hand, some experienced and practical trappers will tell you that the moccasins become so plentiful and take such toll of young that the muskrat population is kept down."

Bibliography

Arthur, Stanley C. 1928 The fur animals of Louisiana. Louisiana Dept. Cons., Bull. No. 18.

Gloyd, Howard K. 1940 The rattlesnakes, genera *Sistrurus* and *Crotalus*. Chicago Acad. Sc., Spec. Pub. No. **4**: 1–266.

Heymons, R. 1935 Pentastomida. In Bronn's Klassen und Ordnungen des Tierreiches, 5, Abt. 4, Lief 1–2: 229–233.

Penn, George H., Jr. 1940 Observations on the life cycle of a linguatulid, *Porocephalus crotali* (Humboldt 1808). Anat. Rec. **78** Suppl.: 125.

Penn, George H., Jr. and Martin, Ernest C. 1941 The occurrence of porocephaliasis in the Louisiana muskrat. J. Wildlife Management **5**: 13–14

Sambon, L. 1922 A synopsis of the family Linguatulidae. J. Trop. Med. **25**: 188–206; 391–428.

THE METACERCARIA AND ADULT OF *CENTROCESTUS FORMOSANUS* (NISHIGORI, 1924), WITH NOTES ON THE NATURAL INFECTION OF RATS AND CATS WITH *C. ARMATUS* (TANABE, 1922)

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In the fall of 1939 when an extensive examination of local fish was made for certain trematode infections a large number of oval metacercariae characterized by double circles of circumoral spines and a very distinct X-shaped excretory bladder was found in the gills of *Macropodus opercularis* and *Puntius semifasciolatus* which were collected from ponds and pools of Tsuen Wan and Fanling, villages between Kowloon and the Chinese-British border in the New Territories of Hong Kong. Feeding of these cysts to white rats resulted in the recovery a week later of many adult trematodes of the species *Centrocestus formosanus* (Nishigori, 1924), a heterophyid reared experimentally in man in Formosa and a potential human parasite in this region.

Mr. C. T. Cheng of this division called the author's attention to similar cysts in the stomach wall and striated muscles of the frog, *Rana limnocharis,* from Kowloon. These likewise proved to be *C. formosanus* when reared in white rats.

HISTORICAL ACCOUNT

Centrocestus was first created by Looss (1899) to include *C. cuspidatus* described by him in 1896 under the name *Distomum cuspidatum.* In 1913 Leiper described as *C. cuspidatus caninus* a fluke from Formosa which is morphologically the same as *C. cuspidatus* but with only 28 spines surrounding the mouth. This name was considered a synonym of *C. cuspidatus* by Ransom (1921). Tanabe (1922) erected a new genus, *Stamnosoma,* for the reception of a new fluke from Japan, *Stamnosoma armatum,* which is closely similar to *Centrocestus.* Following the same generic concept Nishigori (1924) described *S. formosanum* from northern Formosa.

Chapin (1926), Fuhrmann (1928), Witenberg (1929), Price (1932), and Stiles and Baker (1934) all consider *Stamnosoma* to be a synonym

Received for publication, September 22, 1941.

[1] Thanks are due to the following friends for securing certain literature not available in Hong Kong: Dr. C. M. Africa, Dr. H. F. Hsu and Dr. S. M. K. Hu. Mr. S. Y. Lin of the Hong Kong Fisheries Research Station has kindly identified the fish hosts for the writer. To Dr. F. G. Wallace I am in appreciation for assistance in preparing the manuscript.

[2] The University was temporarily located in Hong Kong after November, 1938.

of *Centrocestus*. Faust and Nishigori (1926) accepted the genus *Stamnosoma* and considered that Leiper's specimen of *C. cuspidatus caninus* was really *S. formosanum*. Later, however, Faust (1937) discarded the name *Stamnosoma* in favor of *Centrocestus*.

The metacercarial stages of *C. armatus* and *C. formosanus* have been briefly described by Tanabe (1922) and Nishigori (1924) respectively. Yamaguti (1938) added to our knowledge of the metacercariae and earlier stages of *C. armatus*. *C. formosanus* has been reported from the Philippines by Vazquez-Colet and Africa (1939, 1940) and Africa, de Leon and Garcia (1940). In addition Vazquez-Colet and Africa (1938) report from a marine fish in the Philippines, *Ambassis buruensis* (lañgaray), two apparently different species of *Centrocestus* (one big and having a long intestine, and the other very tiny and having a short intestine). The larger of the two also parasitizes another marine fish, *Hepsetia balabacensis*. The authors consistently failed to infect albino rats and mice, kittens and puppies, indicating apparently that the two metacercariae are distinct forms.

Because of the brevity of extant descriptions the metacercaria of *Centrocestus formosanus* is fully described here and certain obscure morphological aspects of the adult are elucidated.

In China the genus was first found in *Mus norvegicus* in 1933 by the writer and was reported from domestic cats (Chen, 1934), both from Canton.

THE METACERCARIA

The following descriptions are based primarily on material from fish unless otherwise stated, although there are no appreciable differences between these and specimens from frogs.

The cysts are most frequently found attached to the gill filaments, occasionally in the operculum and very rarely in the muscle or on the scales, showing that they prefer tissues in which there is an abundant supply of blood and oxygen. For instance, in a series of 12 *M. opercularis* examined for cysts, one each was found on the scales of 3 fishes, from 1 to 4 on the operculum of 4 fishes, 3 in the muscle of one host, and from 2 to 527 (averaging 126 per fish) on the gills. In frogs they are primarily located in the stomach wall, but may often be found in the muscles.

The cyst is oval and consists of a single-layered, strong, transparent wall about 2 μ thick (Fig. 7). Fifty-two living cysts in water without pressure measured from 0.132 to 0.204 mm (av. 0.176 mm) long and 0.105 to 0.168 mm (av. 0.127 mm) wide. The cyst is always covered by a thick layer of the reaction tissue of the host. With the latter intact 42 cysts gave while alive an average of 0.208 by 0.154 mm.

Excystation can take place easily when the cyst is placed in pepsin digestive fluid for about 2 hours at 37° C and is then placed in pancreatic juice. The thin wall becomes hyaline and swollen to a thickness of about 5 μ, and then is broken by a constant stretching of the parasite. Excysted individuals (Fig. 8) are very active, and it is difficult to measure the body. Under slight pressure the measurements of 8 individuals were 0.267–0.386 mm (av. 0.301 mm) long and 0.099–0.200 mm (av. 0.140 mm) wide. The entire body is studded with short but conspicuous spines. The oral sucker is 0.053 mm wide by 0.058 mm long. The acetabulum is located at about the middle or posterior to the middle of the body and measures 0.033–0.049 mm (av. 0.035 mm) wide and 0.025–0.040 mm (av. 0.031 mm) long. These figures were obtained from 9 specimens.

One of the most characteristic features of this species is the two circles of circumoral spines (Figs. 8, 9), each somewhat recurved at the tip. Those of the outer circle are larger and measure 0.012–0.015 mm long while those of the inner circle are slightly shorter, generally from 0.010–0.012 mm, the width at the base being about 0.002 mm in both. It appears also that those on the ventral side are slightly smaller than the dorsal and are closer together but this point requires more extensive comparisons. The number of spines varies from 30 to 36 with 31 and 32 the commonest. The spines fall away fairly easily and care must be exercised in evaluating their total number as a specific character.

The mouth is large and opens into a funnel-like cavity. The prepharynx (Fig. 8) is practically absent in a very active specimen, but as it becomes sluggish the prepharynx gradually makes its appearance. The average length of the prepharynx of such specimens measured under some pressure of a cover glass is 0.021 mm. The pharynx in 11 specimens measured 0.029 mm wide and 0.033 mm long. Of those which are examined with considerable pressure the pharynx may become wider than long. It is divisible into two parts. The anterior which constitutes the bulk of the structure is muscular, while the posterior third or fourth is granular and is glandular in function. In vitally stained specimens the posterior part is deeply colored while the anterior is colorless (Fig. 12). The esophagus is practically absent in unextended specimens. The average length of 5 was 0.014 mm. The two ceca end either at the posterior border of the acetabulum or between the latter and the anterior border of the excretory bladder. The intestinal ceca may be filled with crystals and are sometimes enlarged posteriorly.

The nervous system consists of a pair of ganglia located on each side of the pharynx or prepharynx and connected by a transverse commissure (Fig. 8). Nerve fibers go upward from each of the ganglia and disappear from view before reaching the oral sucker, while others go downward, cross the ceca in front of the acetabulum, and disappear before reaching the level of the testes.

The reproductive system (Fig. 8) consists of well-developed organs which require only a day or so to become fully developed in the definitive host. The male system consists of two approximately oval testes, slightly lobed at the outer or posterior border, generally placed somewhat obliquely or transversely opposite each other at the posterior end of the body. The left testis measured 0.066 by 0.046 mm and the right 0.065 by 0.460 mm in 10 specimens. Two small ducts lead forward and join together to form the seminal vesicle which is large and consists of two parts divided by a constriction, both located more or less transversely behind the acetabulum. The proximal portion or the posterior vesicle turns from right to left, and the distal portion or the anterior vesicle turns sharply back from left to right. The latter follows the curve of the acetabulum on its lower right border to join the prostate section which is followed by the terminal portion along the anterior right border of the acetabulum. It opens into the common genital atrium in front of the acetabulum. The genital pore can be seen as a transverse slit.

The female system is not as clear as the male system. It consists of a more or less spherical or oval ovary, about half the size of the testes, on the right side just in front of the right arm of the excretory bladder. An oviduct begins from the dorsal side of the ovary, passing transversely to the middle on the ventral side of the seminal receptacle, receiving a short duct from the latter somewhere at the center and connecting with the proximal end of the uterus. Laurer's canal appears to open into the seminal receptacle.

There is a group of banana-shaped skin glands (Figs. 10, 11). Each is connected with a short duct and opens to the outside through the skin. The glands are found all over the body except in the posterior portion. On the ventral side they are arranged in five longitudinal rows. The central row consists of from two to five glands between the oral and the ventral suckers. The lateral rows, each consisting of about 15 glands, extend from below the oral sucker to a level in front of the excretory bladder. The two marginal rows, each consisting of about half a dozen or so glands, occupy the region between the oral and ventral suckers. Variations in this arrangement have been noted.

There is also a group of cephalic glands (Fig. 8) which are crowded together in grape-like aggregations on the sides of the esophagus, the outer margin of the ceca, and between the intestinal bifurcation and the ventral sucker. Some glands are seen between the ventral sucker and the excretory bladder. All the gland cells are granular and do not take vital stains, such as neutral red, Bismarck brown, Nile blue sulphate, or methylene blue. They vary in shape because of crowding.

A complicated system of granular ducts is faintly visible but is extremely difficult to work out (Fig. 8). They are apparently derived

from gland cells at different levels and are massed together at the level of the esophagus and pharynx. At the level of the pharynx the outermost ducts become separated from the main bundle and pass antero-laterally. Immediately anterior to the pharynx all ducts diverge and pass forward dorsally, laterally, and latero-ventrally to the oral sucker to open near the circumoral spines. The ducts are not uniform in diameter but are enlarged here and there so that they appear like strings of beads. They are larger in diameter near the oral sucker.

The excretory system (Fig. 8) is characterized by a very conspicuous X-shaped excretory bladder. From each side of the bladder a convoluted collecting tubule extends upward to the level of the pharynx and then bends down with its diameter much decreased. Its further course cannot be traced. Eight pairs of flame cells were found in all specimens studied. Their arrangement is shown in Fig. 8.

Pigment granules of irregular size are found ventrally and dorsally in the middle region of the body.

FISH HOSTS

In Formosa (Nishigori, 1924): *Carassius auratus* (Linn.), *Clarias fuscus* (Lacépède), *Channa formosana* (Jordan and Evermann), *Pseudorasbora parva* (Schlegel), *Rhodeus occelatus* (Kuer), *Gambusia affinis* (Baird and Girard), *Polyacanthus operculatus* (Linn.), *Ctenopharyngodon idellus* (C. and V.), *Ophicephalus tadianus* (J. and E.), *Misgurnus anguillicaudatus* (Cantor), *Parasilurus asotus* (Linn.), *Zacco platypus* (T. and S.) and *Cyprinus carpio* (Linn.).

In the Philippines (Vazquez-Colet and Africa, 1939): *Ophicephalus striatus* Bloch, *Glossogobius giurus* Buchanan-Hamilton, *Therapos plumbeus* (Kuer) and *Anabas testudineus* (Bloch.); (Vazquez-Colet and Africa, 1940): *Hemiramphus dussumieri* Cuvier and Valenciennes.

In Hong Kong: *Carassius auratus* (Linn.) from Fanling, 63 per cent infected; *Macropodus opercularis* (Linn.) from Fanling 82 per cent and from Tsuen Wan 91 per cent infected; *Puntius semifasciolatus* (Gunther) from Fanling 70 per cent and from Tsuen Wan 93 per cent infected. *Limia caudofasciata* Regan was experimentally infected in the laboratory. The incidence of infection in *Rana limnocharis* Gravenhorst from one locality at Kowloon is less than 2 per cent and from another 25 per cent. The intensity of infection in *Puntius* is from 2 to 288 cysts, with an average of 32 per host, in *Macropodus* 2 to 527 with an average of 128 per host, in *Carassius* 2 to 31 cysts, with an average of 7 per host. In frogs the intensity of infection is from 2 to 40.

In addition *Misgurnus anguillicaudatus* (Cantor), another freshwater fish, and *Bufo melanostictus* Schneider, the common toad, are also infected, their incidence and intensity of infection not being recorded.

TABLE 1.—*Results of feeding experiments with* Centrocestus *cysts from various sources*

Experimental host	Source of cysts	No. of cysts	Days after feeding	Worms recovered	Maturity
Mus rattus	*P. semifasciolatus*	428	4	67	Eggs present
White rat	"	288	31	9	" "
"	"	1010	3	204	" "
"	*Rana limnocharis*	20	2	4	" "
Duckling	*P. semifasciolatus*	278	3	10	" "
"	*M. opercularis*	320	5	A few	" "
Chicken	*C. auratus*	38	3	4	Immature
"	*P. semifasciolatus*	86	8	3	Eggs present
White mouse	"	401	8	35	" "
"	"	407	24	31	" "
"	*M. opercularis*	125	26	4	" "
Rabbit	"	102	15	6	" "
"	*P. semifasciolatus*	197	45	13	" "
Puppy	*M. opercularis*	430	58	7	" "
Kitten	*Puntius* and *Macropodus*	611	11–17	14	" "

THE EXPERIMENTALLY OBTAINED ADULT

Because there are as yet no available descriptions of this species in other languages than Japanese and because there are several structures which require further elucidation, the following description is given, with measurements in millimeters. The metacercaria of this species is very advanced in development, so what has been said concerning this stage is generally applicable to the adult.

The results of feeding experiments with various final hosts are presented in Table 1; sample measurements in Table 3.

Centrocestus formosanus (Nishigori, 1924)

Description: Body flask-shaped, somewhat flattened dorso-ventrally. Twenty specimens measured 0.314–0.572 (av. 0.461) long by 0.171–0.242 (av. 0.212) wide. Oral sucker terminal, 0.056 wide by 0.060 long. Acetabulum at middle of body, 0.055 wide by 0.052 long. Oral sucker armed with two circles of spines somewhat recurved near tip and directed backward, numbering generally 30–32 and occasionally 33–36 (Figs. 1, 2, and 3). Inner circle with larger spines each measuring 0.010 to 0.013 long and 0.002–0.003 broad at base; outer circle with spines 0.008–0.010

TABLE 2.—*Showing the age of the trematode as related to the number of eggs present*

No. of worms	Host	Age in days	No. of eggs present
3	White rat	1.5	0, 2, 3
7	"	3–7	3, 4, 4, 5, 6, 8, 16
14	"	10	2, 6, 9, 9, 10, 11, 11, 12, 13, 14, 17, 20, 25, 28
3	"	12	21, 28, 32
2	Rabbit	15	4, 4
8	Kitten	11–17	1, 11, 12, 12, 15, 18, 18, 24
9	Mouse	25	1, 2, 2, 3, 3, 5, 5, 5, 7
2	White rat	30	20, 20
6	Rabbit	45	16, 17, 18, 21, 23, 48
5	Puppy	58	10, 17, 22, 22, 25

TABLE 3.—*Measurements of various parts of the two specimens from rats. In millimeters*

	Specimen 1	Specimen 2
Body	0.598 by 0.360	0.583 by 0.274
Oral sucker	0.088 wide	0.060 wide
Prepharynx	0.028 long	0.053 long
Pharynx		0.046 wide
Testes	0.123 by 0.077	0.105 by 0.060
Ovary		0.063 diameter
Seminal receptacle	0.122 by 0.086	0.098 by 0.070
Vitellaria	From level of prepharynx to posterior end of body	Same as in specimen 1
Eggs	0.035 by 0.023. Over 60 eggs altogether	0.034 by 0.024. As many as in specimen 1

long and about 0.002 broad at base. In some specimens smaller chitinous rodlets, or sometimes true spines, either singly or in pairs, may be found irregularly between spines of outer or inner circle. In specimens one month or more old, spines are larger, inner being 0.019 by 0.005 and outer 0.016 by 0.003, and tips are mostly worn off.

Mouth opens into prepharynx which is about 0.072 long by 0.032 wide. Pharynx 0.42 wide by 0.041 long, and followed by very short esophagus. Ceca large and extend to level of middle of ovary.

Two testes, oval, slightly lobed, on posterior border, right 0.106 by 0.071 and left 0.101 by 0.074, placed directly opposite each other at posterior end of body. One duct goes forward from each testis and the two

TABLE 4.—*Measurements in microns of* Centrocestus armatus, C. cuspidatus *and* C. formosanus

	C. armatus (From Tanabe)	*C. cuspidatus* (From Looss)	*C. formosanus* (From Nishigori)	*C. formosanus* (From Chen, this article)
Body	456 × 236	500 × 350	362 × 197	461 × 212
Oral sucker (length and width)	64 × 68	Diam. 50	50 × 47	60 × 56
Oral spines Number	44	36	32	30 – 36
Oral spines Size	36 × 19.6	10 × 3	Outer circle: 14 × 2.2 Inner circle: 12.5 × 1.7	Outer circle: 13 × 2 young 19 × 5 old Inner circle: 8 × 2 young 16 × 3 old
Acetabulum (length and width)	50 × 61	Diam. 60	36–43 × 39–55	52 × 55
Pharynx (length and width)	43.8 × 34	30 × 25	40–47 × 20–35	41 × 42
Testes Right	110 × 60	150 × 80	89 × 43	106 × 71
Testes Left	103 × 60		78 × 42	101 × 74
Ovary	Lobed posteriorly 95 × 65		Lobed 54 × 40	83 × 67
Seminal receptacle	78 × 61	Diam. 70	53 × 50	80 × 62
Seminal vesicle	286 × 46.5	Large	Ant. 122 × 31 Post 81.2 × 16	Ant. 78 × 35 Post. 69 × 37
Eggs Number	Several tens to over 100	About 50	Over 10 to 45	About 20
Eggs Size	31 × 17	30 × 15 (embryonated)	33 × 17	33 × 16

meet to form large seminal vesicle constricted at about middle into two sausage-shaped parts, posterior portion being 0.037 by 0.069 and going obliquely outward and anterior being 0.035 by 0.078 and going almost transversely inward by following curve of acetabulum. At distal end it narrows considerably and curves upward along right corner of acetabulum, where it joins prostate region on right border of sucker, this portion being surrounded by several unicellular structures, the prostate glands. Terminal portion or ejaculatory duct opens into common genital atrium in front of acetabulum (Fig. 4). In center of the atrium opens genital pore which is transverse slit.

Ovary oval, slightly lobed, measuring 0.083 by 0.067, in front of right or occasionally of left testis. Oviduct passes more or less transversely to middle and receives a short duct from round or oval seminal receptacle, which averages 0.080 by 0.062, and is located in the middle of the body just in front of testes. Oviduct next receives duct from vitelline reservoir and then passes on as an oötype surrounded by Mehlis' glands. Laurer's canal opens apparently into seminal receptacle near junction of oviduct. Uterus bends several times between anterior border of testes and level of genital pore. It contains from 10 to 30 eggs (see Table 2). Metraterm extends upward along curve at left of acetabulum to open into common genital atrium (Fig. 4). Common vitelline duct is just in front of testes and reservoir is somewhat triangular in shape. Vitellaria form spherical follicles located outside of ceca and other organs and extending from lower level of pharynx to region behind testes at posterior end of body.

Eggs show characteristic latticed designs on outside. They measure 17 to 20 μ wide and 32 to 39 μ long with average of 19 by 34 μ. Shape is typically that of chicken egg. Egg shell 1.4 μ thick, uniform throughout; operculum 6 μ wide. Fig. 5 shows most of above characters. Occasionally another type of eggs may be found. These are apparently either first few eggs or are unfertilized. They are narrower and longer, 17 μ by 43 μ, with less conspicuous latticed design (Fig. 6). Egg shell is slightly thinner and often provided with nodule placed slightly to one side at posterior pole of the egg.

Nervous and excretory systems, as far as studied, are as in the metacercaria.

Definitive Hosts: In Formosa: *Nycticorax nycticorax* and dogs, and experimentally in cats, white rats, mice, rabbits, etc. In the Philippines: *Bubulcus ibis coromandus* and *Pyrreroides manilensis,* and experimentally in dogs and cats. In Hong Kong: experimentally in white rats, white mice, chickens, *Mus rattus,* rabbits, ducklings, puppies and kittens.

Location in Host: Small intestine.

NATURAL INFECTION IN RATS AND CATS[3]

Two worms of this genus were collected from rats, *Mus norvegicus,* in 1933 in Canton but were poorly preserved. Table 4 gives measurements of structures that could be made out from these worms.

The measurements were based on permanent mounts in which, due to some pressure from the cover glass, the size is somewhat exaggerated but the relationships of different organs remain constant. The circumoral spines are distinct but their number cannot be made out. The eggs do not have latticed design on the outside. Because of this and the larger number of eggs the writer is inclined to call this *Centrocestus armatus* tentatively.

The single worm that was collected from a cat in Canton (Chen, 1934) was much like the two from rats and before more can be collected it is placed in the same species.

DISCUSSION

The differentiation of the three known species of *Centrocestus* has been based chiefly on the number of circumoral spines, the form of the margin of the testes and ovary, and the presence or absence of the latticed design on the eggs (see Table 4 for detailed comparison). The number of circumoral spines appears to be rather definite in the three species according to the various authors. Thus in *C. cuspidatus* Looss gave 36 spines, in *C. armatus* Tanabe mentioned 44, and in *C. formosanus* Nishigori counted 32. In the present study, however, it has been shown that in the last species the number of the spines varies from 30–36, with 30–32 being the commonest. In *C. armatus,* Yamaguti in 1933 observed from 40–44 spines and in 1938 only 40. Moreover, it has been noted that spines may easily fall off, and if this happens near the lateral corner of the crown where they are more closely arranged, it will be very difficult for one to detect such an anomaly. These observations indicate that it is not entirely reliable to depend on the number of the spines as a specific character unless a large number of specimens are carefully studied.

With reference to the form of the margin of the testes and ovary it is noted that in *C. cuspidatus* both of these organs have smooth margins while in *C. armatus* and *C. formosanus* they are lobed. In the present study it has been observed that if specimens were left in the physiological salt solution there would be no change in the outline of these two organs, but if they were placed in water the two organs would become smooth. It is well, therefore, to be cautious in considering such characters as of specific importance.

Eggs of the several species are apparently similar in size and shape.

[3] Three specimens from these hosts were collected from Canton but all were lost in transit to Hong Kong in the fall of 1938.

Three points, however, require special attention. The first is the latticed design in *C. formosanus*. It is a character which is very important but may be easily overlooked. The question as to whether such a design is present in the eggs of other species cannot be answered, of course, without seeing the specimens. The earlier suggestion that *C. cuspidatus caninus* is a synonym of *C. formosanus* must be verified by a study of the egg shell of the former. The second point of interest is the presence of an almost fully developed embryo in the egg of *C. cuspidatus* according to Looss. This is not true with the two other species. The third point of interest is the number of eggs in a fully matured specimen. Tanabe gave several tens to over a hundred eggs for *C. armatus* and Nishigori gave from over ten to as many as 45 for *C. formosanus*. In *C. cuspidatus* Looss did not mention the number, but from the drawing there are roughly 50 eggs. It is likely that this is not the true number but it shows that they are numerous. Unquestionably the number of eggs depends to a certain extent on the age of the parasite, but this must be within a reasonable limit.

In the present study the age of the fluke as related to the number of eggs present in the body at the time of the autopsy of the host has been subjected to a careful scrutiny. Table 2 shows the result. Among the white rats the number of eggs is over 20 when the parasite is more than 10 days old. Thus in the three flukes of 12 days old, 21, 28 and 32 eggs are found respectively, and in the two flukes of 30 days old, 20 eggs are found in each. In other hosts listed in the table, variations are somewhat greater in the direction of smaller number of eggs. This is particularly true in the 15-day infection in a rabbit in which only four eggs are present in each of the two worms studied, and in the 25-day infection in a mouse in which the number of eggs present in the 9 specimens studied varied from one to 7. Only one worm possesses more than 32 eggs, i.e., the one in a rabbit of 45-day infection in which one of the parasites possesses 48 eggs, the maximum found so far. This must be considered unusual.

This leads us next to a consideration of the definitive host of this parasite. Which is the normal host? The data available from various sources are not sufficient for a positive answer at the present time. As noted in a previous paragraph *Nycticorax nycticorax* and dogs are naturally infected in Formosa, and *Bubulcus ibis coromandus* and *Pyrreroides manilensis* in the Philippines. This does not necessarily mean that they are the optimum hosts. Natural hosts of this parasite in China have not yet been reported. On the basis of experimental data as listed in Table 1 one can readily see that rats are the most favorable hosts as the percentage of worms recovered from each of them is greater than in other hosts. Nevertheless, the number of viable cysts fed to the rats is several times the

number of adults recovered from them. This shows that probably rats are not the optimum hosts.

There is also some indication that a certain amount of acquired immunity may be accumulated, since fewer worms are recovered in infections of longer standing (see Table 2). More intensive study is needed on this phase of the subject.

Attention must also be called to structures like the prepharynx and esophagus which are not only variable but may also be present or absent in specimens depending upon the state of contraction in which they are fixed. In a flattened or relaxed specimen both of these structures are present and sometimes rather long. On the other hand if they are active or killed and fixed when first separated from the host these two structures are either absent or only slightly visible. In a somewhat flattened specimen the prepharynx may be long and slender when the body is extended, or short when the body is contracted. Oftentimes the prepharynx cannot simultaneously contract with the body, but becomes more or less bent to one side forming a pocket. It straightens out immediately as soon as the body is again stretched. Occasionally at the contraction of the body the prepharynx expands on all sides at its lower part so that from ventral or dorsal views it looks as if two prepharyngeal pockets are formed one on each side as seen in the picture of *C. cuspidatus* (Looss, 1896). Due to the varying position of the prepharynx and the pharynx the use of the position of the ganglia as a diagnostic character to distinguish *C. cuspidatus* from *C. armatus* as maintained by Yamaguti will be of no value.

The brief discussion shows that eggs possess clear-cut characteristics and are most dependable for distinguishing the three species. The status of *C. cuspidatus caninus* cannot be settled until its eggs are re-studied. The circumoral spines are important but their variation in number must be borne in mind. Other structures are too variable and cannot be depended on. Table 4 is a compilation of some characters of the various species of *Centrocestus* given by various workers.

The finding of metacercariae of *C. formosanus* in frogs is of considerable importance. From the biological standpoint it shows that heterophyid metacercariae, which have hitherto been known to parasitize only fish in this stage, can also infect other hosts of an entirely different group. The discovery makes it necessary to modify the diagnoses of the family HETEROPHYIDAE Odhner, 1914, and the sub-family CENTROCESTINAE Looss, 1899.

From the public health standpoint the discovery is of additional interest if further work can show that any edible species of frogs, such as *Rana rugulosa,* is involved in this region. Nishigori has already shown that this species is transferable to man. In this region, the only fishes so far involved are small and not ordinarily used as food but if edible

frogs are involved another possible source of human infection exists. The writer has good reason to believe that *R. rugulosa* may be infected and investigation is being carried on in this direction. Considering that frog meat is most delicious when cooked in a "lightning" manner one can easily imagine its potential danger. Perhaps *Centrocestus* in man is overlooked because the parasites are very small and the eggs are similar to commoner species of human flukes, such as *Clonorchis sinensis.*

SUMMARY

The metacercaria of *Centrocestus formosanus* is described in detail for the first time. The parasites encyst chiefly in the gills of four freshwater fishes, namely, *Macropodus opercularis, Puntius semifasciolatus, Carassius auratus,* and *Misgurnus anguillicaudatus,* and in the stomach wall and muscles of a common frog, *Rana limnocharis,* and a common toad, *Bufo melanostictus.* The experimentally obtained adult together with its eggs is also treated in detail.

As a result of present observations the eggs are believed to be of importance in specific diagnosis of the three known species. The number of circumoral spines is of value within certain limits because it varies. Other morphological structures appear to be undependable for specific differentiation of the genus.

Worms from natural infections of *Mus norvegicus* and a cat from Canton are believed to be *C. armatus.*

The importance of *C. formosanus* from the public health aspect is briefly discussed.

BIBLIOGRAPHY

AFRICA, C. M., DE LEON, W. AND GARCIA, E. Y. 1940 Visceral complications in intestinal heterophyidiasis of man. Acta Med. Philippina No. 1, pp. 1–132.

CHAPIN, E. A. 1926 Note on the Heterophyidae. J. Parasitol. **12**: 180.

CHEN, H. T. 1934 Helminths of cats in Fukien and Kwangtung Province with a list of those recorded from China. Lingnan Sc. J. **13**: 261–273.

FAUST, E. C. 1937 Helminths and helminthic infections. In Craig and Faust's "Clinical Parasitology," Philadephia, pp. 217–474.

FAUST, E. C. AND NISHIGORI, M. 1926 The life cycles of two new species of Heterophyidae. J. Parasitol. **13**: 91–128.

FUHRMANN, O. 1928 Trematoda, in Kuekenthal's "Handbuch der Zoologie," Berlin and Leipzig, **2**: 1–140.

LEIPER, R. T. 1913 Seven helminthological notes. J. London School Trop. Med. **2**: 175–178.

LOOSS, A. 1896 Recherches sur la faune parasitaire de l'Egypt. I. Mem. Inst. Egypt **3**: 1–296.

————1899 Weitere Beiträge zur Kenntniss der Trematoden-Fauna Aegyptens. Zool. Jahrb. Syst. **12**: 521–784.

NISHIGORI, M. 1924 On a new species of fluke, *Stamnosoma formosanum,* and its life history. Taiwan Igakkai Zasshi No. 234: 181–238. (Japanese, with English summary.)

PRICE, E. W. 1932 On the genera *Centrocestus* Looss and *Stamnosoma* Tanabe. J. Parasitol. **18**: 309.

RANSOM, B. H. 1921 Synopsis of trematode family Heterophyidae with descriptions of a new genus and five new species. Proc. U. S. Nat. Mus. **57**: 527–573.

STILES, C. W. AND C. E. BAKER. 1934 Key-catalogue of parasites reported for Carnivora (cats, dogs, bears, etc.) with their possible public health importance. Bull. Nat. Inst. Health, U. S. Pub. Health Serv., No. 163, p. 945.

TANABE, H. 1922 Studien ueber die Trematoden mit Suesswasserfischen als Zwischenwirt. 1. *Stamnosoma armatum* n. g., n. sp. Kyoto Igaku Zasshi **19**: 1–14. (Japanese, with German abstract.)

VAZQUEZ-COLET, ANA AND C. M. AFRICA 1938, 1939 Determination of the piscine intermediate hosts of Philippine heterophyid trematodes by feeding experiments. Philippine J. Sc. **65**: 293–302; **70**: 201–215.

——— AND ——— 1940 Morphological studies on various heterophyid metacercariae with notes on the incidence, site, and degree of metacercarial infection in three species of marine fish. Philippine J. Sc. **72**: 395–420.

WITENBERG, A. 1929 Studies on the trematode family Heterophyidae. Ann. Trop. Med. and Parasitol. **23**: 131–239.

YAMAGUTI, S. 1933 Studies on the helminth fauna of Japan. Part 1. Trematodes of birds, reptiles and mammals. Japan. J. Zool. **5**: 1–134.

———1938 Zur Entwicklungsgeschichte von *Centrocestus armatus* (Tanabe) mit besonderer Beruecksichtigung der Cercarie. Z. Parasitenk. **10**: 293–296.

EXPLANATION OF PLATE, p. 298

Centrocestus formosanus (Nishigori, 1924)

FIG. 1. Adult of *Centrocestus formosanus.*

FIG. 2. Oral sucker of adult showing circumoral spines.

FIG. 3. Oral sucker of adult showing circumoral spines and rodlets.

FIG. 4. Acetabular region of adult showing anterior portion of seminal vesicle, prostate region, ejaculatory duct, metraterm, vagina, and genital pore.

FIG. 5. Egg showing characteristic latticed design of shell.

FIG. 6. Abnormal egg of unusual length.

FIG. 7. Encysted metacercaria.

FIG. 8. Excysted metacercaria.

FIG. 9. Circumoral spines of metacercaria.

FIG. 10. Ventral view of excysted metacercaria showing arrangement of skin glands.

FIG. 11. Excysted metacercaria showing another arrangement of skin glands.

FIG. 12. Pharynx showing glandular and muscular portions.

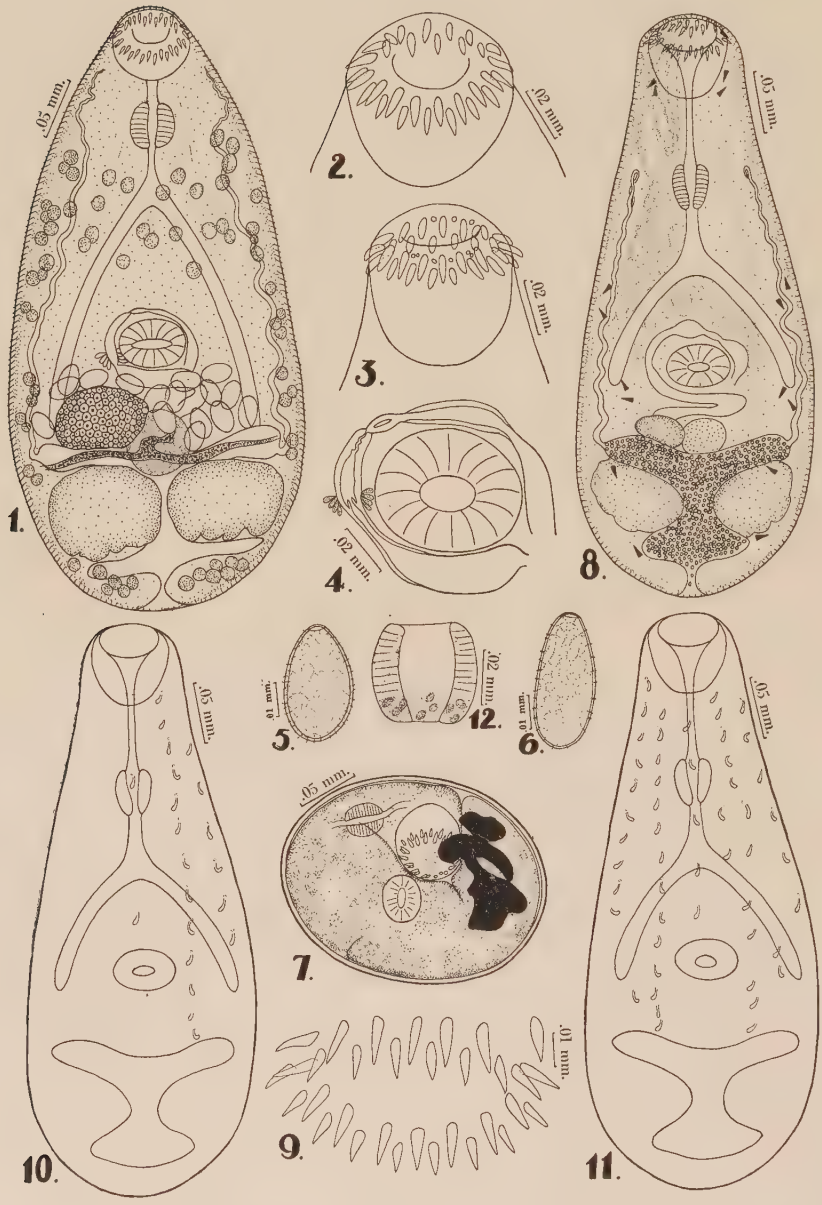
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PLATE I

OBSERVATIONS ON THE MACROSCOPIC SPECIES-IDENTIFICATION OF LARVAL *ANOPHELES* IN GEORGIA*

R. Edward Bellamy

In most places where routine surveys of local *Anopheles* distribution have been undertaken by large scale collection and identification of larvae from nearby aquatic situations, a certain "familiarity" with the larvae has probably developed. The familiarity, in some cases, has been developed into a technique for distinguishing the larvae of the local species by their macroscopic appearance. Dr. W. V. King, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, related (in lectures to malaria entomology trainees at Orlando, Florida, 1937) that Philippine assistants learned in their country to distinguish macroscopically, with a high degree of accuracy, the larvae of different species of *Anopheles* occurring there. Dr. Arnaldo Gabaldon, Division de Malariologia, Direccion de Salubridad Publica, Ministerio de Sanidad y Asistencia Social, Caracas, Venezuela, stated (in personal communication) that, in Venezuela, sanitary inspectors may learn to distinguish macroscopically the larvae of anopheline species encountered in a small area but that this ability is lost if they are transferred to a new area. Dr. D. Bruce Wilson states (in a manuscript report to the International Health Division of the Rockefeller Foundation, 1940) that in the campaign against *Anopheles gambiae* in Brazil, a technique was developed for distinguishing macroscopically the larvae of this species from larvae of the Brazilian *Anopheles* of the subgenus *Nyssorhynchus*. D. Manson (1934) has tabulated certain macroscopically visible characters of the larvae of eighteen species of Indian *Anopheles* as an aid to the macroscopic identification of these forms, and states that with a short period of training and attention to detail one may learn to distinguish larvae of the more common species of Assam *Anopheles* "with a considerable degree of accuracy."

A few months after inauguration of *Anopheles* survey work in South Georgia, it was noted that the presence or absence of larvae of *Anopheles quadrimaculatus* in collections was usually correctly anticipated in the

Received for publication, October 4, 1941.

* Contribution No. 5 from the Division of Malaria and Hookworm Service, Georgia Department of Public Health, Atlanta, Georgia. Read in part, before the National Malaria Committee and the American Society of Tropical Medicine at Louisville, Kentucky, on November 14, 1940. For their invaluable assistance in connection with this study, I wish to thank Dr. Justin Andrews, Mr. R. S. Howard, Jr., Dr. Glendy G. Sadler, Dr. Horace O. Lund, Mr. Alan Donaldson, and Dr. H. E. Savely, Director, Engineer, and one time Biologists, respectively, of the Division of Malaria and Hookworm Service, Georgia Department of Public Health; and Mr. Melvin H. Goodwin, Jr., Biologist, and Mr. John Platt, Summer Biologist, of the Emory University Field Station, Baker County, Georgia.

field, and that in mixed collections of *A. quadrimaculatus* and *A. crucians* the specimens of the two species could be separated rather successfully "by eye" before the routine determinations by microscope. Larvae of *A. punctipennis* were not found in abundance during this period, and (as microscopic determinations revealed) were "by eye" often mistaken for larvae of *A. quadrimaculatus* and occasionally for larvae of *A. crucians*. Because of the difficulty of distinguishing by eye *certain* larvae of *A. punctipennis* from *certain* larvae of the other two common species, it appeared that mistakes in macroscopic identification might be reduced to a minimum if these "difficult" larvae could be separated by eye as such, and only the remainder of the larvae identified macroscopically to species.

After about a year of field experience, a program was inaugurated to check against microscopic determinations the macroscopic identification of all routine collections of fourth-instar *Anopheles* larvae. At the outset of the study, it was decided that each fourth-instar *Anopheles* larva taken in routine work was to be assigned from its macroscopic appearance (and before examination by microscope) to one of the following groups or designations:

Group 1. Larvae macroscopically identified as *A. quadrimaculatus*.

Group 2. Larvae macroscopically identified as *A. crucians*.

Group 3. Larvae macroscopically identified as *A. punctipennis*.

Group 4. Larvae macroscopically "difficult" but considered from macroscopic appearance to be either *A. quadrimaculatus, A. punctipennis,* or *A. walkeri*.

Group 5. Larvae macroscopically "difficult" but considered from macroscopic appearance to be either *A. crucians, A. punctipennis,* or *A. georgianus* (*Anopheles georgianus* was recognized as an entity at the time (Bellamy, 1939) although it was not described until later (King, 1939) and subsequently raised to specific status (King and Bradley, 1941)).

Group 6. Dead and injured fourth-instar larvae.

The "difficult" larvae were to be assigned to groups 4 and 5, but here also a partial separation was attempted in that no provision was made for *A. crucians* in group 4 and none was made for *A. quadrimaculatus* in group 5. As dead and badly injured larvae do not exhibit the characteristics which aid in macroscopic identification, no attempt was made to identify these by eye.

Microscopic determinations to check all macroscopic identifications were planned as routine.

No provision was made for recording the macroscopic identification of *Anopheles barberi,* but with the peculiar appearance and habitat of this species little or no doubt has existed as to the identity of specimens whenever they have been encountered and, as only special collections from

brackish-water areas contained specimens of *A. atropos* and *A. bradleyi,* no provision was made for macroscopic identification of these larvae.

PROCEDURE

The collected fourth-instar larvae of *Anopheles* were brought from the field to the laboratory and there on examination by the unaided eye were assigned to the various groups explained above. The larvae were then placed in individual depressions in a porcelain color plate and were determined by the recognized microscopic characteristics (Bradley, 1936) (chaetotaxy) with a binocular dissecting microscope. On microscopic determination, the larvae were recorded by number of each species under the macroscopic designations (groups) as assigned. In almost all cases the microscopic determinations were accepted as final. Occasional larvae were reared as supplementary check, but in no case was the identification of larva by microscope reversed. During the first year of this study, the collected first-, second-, and third-instar larvae were reared to the fourth larval instar and then treated as the larvae which were fourth instar at first examination of the collections.

MACROSCOPIC IDENTIFICATION OF ROUTINE SURVEY COLLECTIONS

In the preliminary recorded check of macroscopic recognition of the specific identity of larval *Anopheles,* 821 living and 10 dead or injured fourth-instar larvae were examined in 48 collections from as many different ponds and swamps in Calhoun County, Georgia. Almost immediately following the preliminary test in Calhoun County, a similar test was made on collections in Burke County, Georgia, with very similar results obtaining, although the two counties are almost 200 miles apart. Following these tests, only sporadic attention was given to macroscopic identifications. Thus, in a period of more than a year, further macroscopic identifications were checked on only 2460 fourth-instar larvae in 157 collections from 15 different Georgia counties, though several hundred collections were made during this period.

The results of the macroscopic identification of these larvae are presented in Table 1. It may be observed that in the collections from 15 different counties, larvae of *Anopheles punctipennis* predominated and a proportionately higher error was made in the macroscopic identification of larvae in these collections than was made in identification of the Burke and Calhoun County collections in which relatively few larvae of *A. punctipennis* were encountered.

Summary of the Calhoun, Burke, and miscellaneous collections (Table 1) shows that macroscopic identification of 4217 routinely collected fourth-instar anopheline larvae was attempted. Two hundred ninety-nine or 7.1% of these larvae were assigned to the "difficult" groups,

while 3911 (92.9%) were macroscopically identified to species with only a 1.1% error, a macroscopic misidentification of 45 specimens. The error was distributed as follows: of 1570 larvae called *A. quadrimaculatus,* an error of 1.0% (15 specimens) was made; of 1062 larvae called *A. punctipennis,* an error of 2.5% (27 specimens) occurred; and of 1279 larvae called *A. crucians,* an error of 0.2% (3 specimens) was made.

With less than two months of field experience with anophelines, Dr. G. G. Sadler, then a Biologist in the Division of Malaria and Hookworm Service of the Georgia Department of Public Health, attempted macroscopic identification of the 747 fourth-instar larvae taken in his first 70 collections in Burke County, Georgia. The results are presented with his permission and show that the macroscopic identification can be sufficiently learned in a short time to permit a relatively good record in the identification of larval *Anopheles.*

With the exception of one larva which was placed in a "difficult" group, all larvae were assigned by eye to group 1 (*A. quadrimaculatus*), group 3 (*A. crucians*), or group 6 (dead and injured larvae). It is significant that 23 of the 28 *A. punctipennis* which were taken in these collections were recorded in group 6 while only 31 of 377 *A. quadrimaculatus* and 56 of 333 *A. crucians* were placed there. Macroscopic identifications were quite obviously attempted on only those larvae which appeared either to be *A. quadrimaculatus* or *A. crucians.*

In calling 637 larvae from their macroscopic appearance, Dr. Sadler made an error of less than 6.5% although his previous experience with anophelines consisted of only three weeks of study with the writer and another month of individual study. The identifications are analyzed in Table 1.

IMPORTANCE OF KNOWING THE HABITAT FROM WHICH LARVAE WERE COLLECTED

There is little doubt that knowledge of the field conditions obtaining where the various collections of larvae on which the above tests of macroscopic identification were made was of considerable assistance toward the correct macroscopic identification of the larvae. In order to evaluate the significance of the habitat-association factor, seven malaria workers (Dr. H. O. Lund, Dr. H. E. Savely, Dr. G. G. Sadler, and Mr. Alan Donaldson, Summer Biologists, and Mr. R. S. Howard, Jr., Malaria Investigations Engineer, all of the Division of Malaria and Hookworm Service, Georgia Department of Public Health; and Mr. M. H. Goodwin, Jr. and Mr. John Platt, Biologist and Summer Biologist, respectively, of the Emory University Field Station, Newton, Georgia) variously experienced in malaria entomology, assisted the writer in a wholesale collection of anopheline larvae. The men separated into four groups and the col-

TABLE 1.—*Distribution (determined by microscope) of macroscopically identified 4th-instar larval* Anopheles

	"Definite" groups												Proportion assigned to groups 1, 2, & 3.	Error made in groups 1, 2, & 3.	"Difficult" groups								Group 6	Total larvae
	Group 1 (*A. quad.*)				Group 3 (*A. punc.*)				Group 2 (*A. c .cruc.*)						Group 4				Group 5					
	quad.	*punc.*	*cruc.*	*walk.*	*punc.*	*quad.*	*cruc.*	*walk.*	*cruc.*	*punc.*	*quad.*	*walk.*			*quad.*	*punc.*	*walk.*	*cruc.*	*cruc.*	*punc.*	*georg.*	*quad.*	Dead larvae	
Routine collections																								
Calhoun County 48 collections	532	2	1	0	70	0	3	0	173	0	0	0	95.1%	0.8%	15	7	0	3	11	1	0	3	10	831
Burke County 55 collections	422	0	0	0	45	0	0	0	417	0	1	0	94.5%	0.1%	19	12	1	7	11	1	0	0	70	1006
157 collections from 15 counties ..	601	12	0	0	927	17	7	0	686	1	1	0	90.8%	1.7%	79	75	0	8	28	9	8	1	510*	2970
Totals of above	1555	14	1	0	1042	17	10	0	1276	1	2	0	92.9%	1.1%	113	94	1	18	50	11	8	4	590	4807
70 Burke colls. (G. G. Sadler)† ...	340	4	20	9	0	0	0	0	257	1	5	0	85.3%	6.5%	0	0	0	0	0	0	0	1	110	747
Mixed Series																								
Macroscopically identified by:																								
The writer	403	8	10	0	280	14	2	1	308	3	0	1	97.8%	3.8%	8	3	1	3	5	1	0	2	0	1053
Subject No. 1	406	63	63	1	224	18	42	0	221	6	2	2	99.5%	18.8%	1	2	0	2	0	0	0	0	0	1053
Subject No. 2	320	55	9	1	194	55	95	0	220	33	32	2	96.5%	27.6%	20	11	0	2	2	2	0	0	0	1053

* Includes (with dead larvae) collected young instar specimens which were reared to fourth instar and then identified by microscope only.
† Since Dr. Sadler lumped all "difficult" larvae with dead larvae in Group 6, all larvae in Group 6 have been scored as "difficult" in computing percentage assigned to first three groups; elsewhere Group 6 has been ignored.

lections were made at varied habitats in four different counties in order that there might be, as nearly as possible, an equal and diversified representation of the three common species. The larvae were collected on two successive days and, when brought to the laboratory, were thoroughly mixed.

After completion of these collections, a person inexperienced in macroscopic identification of anopheline larvae pipetted representative fourth-instar specimens individually from the assorted lot to numbered depressions in porcelain color plates. Eight plates were used and each plate had twelve depressions; thus, with one larva in each depression, 96 specimens were available at one time for macroscopic identification. Working independently, the writer and the three of the malaria workers mentioned above who had had some experience in the techniques of macroscopic identification applied themselves to the identification and recording of these sets of larvae by the unaided eye, while the other workers, as the plates of larvae were rotated to them, determined the same larvae by microscope and recorded the results. The macroscopic identifications were checked against the determinations by microscope after all the identifications were complete on the series of 96 larvae. These specimens were then discarded and a second series of larvae was similarly selected, identified, and checked. In this manner eleven series of larvae, totalling 1056 specimens, were checked. The four malaria workers who had had little or no experience in the techniques of macroscopic identification alternately attempted macroscopic identification of about 200 larvae each.

According to the determinations by microscope, the 1056 fourth-instar larvae consisted of 328 *A. crucians,* 427 *A. quadrimaculatus* (including 2 doubtful specimens), 295 *A. punctipennis* (including 12 doubtful specimens), 3 *A. walkeri,* and 3 specimens which were either *A. quadrimaculatus* or *A. punctipennis.* These last three failed to rear to adults as check and were scored as follows: if in the macroscopic identifications they were designated either as *A. quadrimaculatus* or as *A. punctipennis* they were subtracted from the total and were not considered —otherwise they were counted in the total as mistakes.

The writer assigned 2.2% of the larvae to difficult groups and made a 3.8% error in the macroscopic identification of the other 1030 specimens (a misidentification of 39 larvae). The errors made by the other workers ranged from 18.8% to 55.3%. The analysis of these macroscopic identifications (for the writer and two other workers) is shown in Table 1.

The fact that the error made in the macroscopic identification of the mixed series of larvae (3.8%) was greater than that made in identification of routine field collections indicates that the habitat-association factor is of some assistance to correct macroscopic identification. The charac-

teristics of service in macroscopic identification of larvae, however, are most distinct when specimens are in the field or have only recently been brought from the field (a fact also observed by Manson (1934)), and some of the specimens in the mixed series had been collected two days before macroscopic identification of them was attempted. This must account in part for the higher error in the identification of the assorted larvae.

MACROSCOPIC IDENTIFICATION OF PUPAE AND THIRD-INSTAR LARVAE

Immediately following the macroscopic identification of the 1056 fourth-instar larvae, all third-instar larvae which were present in the large mixture of collections were separated by eye into three groups, were placed in three jars, marked *"A. quadrimaculatus," "A. punctipennis,"* and *"A. crucians,"* respectively, and were fed yeast; no specimens were assigned to "difficult" groups. All of the pupae present in the mixture of collections were similarly sorted into three jars marked like those for the third instar larvae; provision was made for the retention of adults as they emerged. Subsequently, Mr. M. H. Goodwin, Jr. identified the pupae as emerged adults and the larvae by microscope as the latter transformed to fourth instar. According to Mr. Goodwin's observations, the results of the macroscopic identification of the third-instar larvae were as follows:

Of the 115 third-instar larvae macroscopically designated as *A. quadrimaculatus* 70 reared to fourth-instar *A. quadrimaculatus,* 5 to fourth-instar *A. punctipennis,* and 2 to fourth-instar *A. crucians,* while 2 were unidentified and 36 died as third-instar.

Of the 88 third-instar larvae macroscopically designated as *A. punctipennis* 72 reared to fourth-instar *A. punctipennis,* 1 to fourth-instar *A. quadrimaculatus,* and 3 to fourth-instar *A. crucians,* while 12 died as third-instar larvae.

Of the 186 third-instar larvae macroscopically designated as *A. crucians* 70 reared to fourth-instar *A. crucians,* 2 to fourth-instar *A. punctipennis,* and none to fourth-instar *A. quadrimaculatus,* while 4 were unidentified and 110 died.

If the larvae which died as third-instar are disregarded and the six larvae which were not identified by microscope are counted as mistakes, an 8.2% error was made in the macroscopic identification of 231 third-instar larvae from the assorted lot; if the six unidentified larvae are disregarded along with those which died as third-instar, the error is less than 5.8%.

From the pupae, Mr. Goodwin identified reared adults as follows:

Pupae macroscopically identified as *A. quadrimaculatus* reared to 88 *A. quadrimaculatus,* 14 *A. crucians,* and 13 *A. punctipennis* adults.

Pupae macroscopically identified as *A. punctipennis* reared to 25 *A. punctipennis,* 21 *A. quadrimaculatus,* and 31 *A. crucians* adults.

Pupae macroscopically identified as *A. crucians* reared to 36 *A. crucians,* 13 *A. quadrimaculatus,* and 9 *A. punctipennis* adults.

The error in the attempted macroscopic identification of pupae was 40.4%, somewhat better than chance but an exceedingly large error.

Previous checks of macroscopic identification of pupae and third-instar larvae gave similar but much better results in the case of the pupae and somewhat better results in the case of the third-instar larvae. However, in these earlier checks knowledge of the identity of the fourth-instar larvae in the collections with the pupae and third-instar larvae undoubtedly served as a valuable guide to the correct guessing of the identity of these latter.

While the experience in macroscopic identification has largely consisted of identification of collections of larvae from southwest Georgia counties, the macroscopic method has been checked against collections of anophelines from Georgia counties bordering the Atlantic Ocean, South Carolina, Tennessee, Alabama, and Florida. No significant difference was noted in the macroscopic appearance of larvae from these different counties.

CHARACTERISTICS EMPLOYED IN MACROSCOPIC IDENTIFICATION

The Table of Characteristics (Table 2) given here is the result of impressions from three years of observations of *Anopheles* larvae and does not represent results of tabulations of numbers of specimens of the different species in the different color phases, etc. Indeed, the gradations in coloration, arrangement of the white dorsal spotting, and the other characteristics would apparently form such a complete series of varieties that an attempted classification on which to base a tabulation of specimens would be exceedingly difficult to establish or, once established, to follow.

As already indicated, a knowledge of the type of habitat from which larvae are taken helps much in making decisions as to their identity. Most helpful of all, certainly, is familiarity with the larvae—the more different "macroscopic types" of larvae with which one is familiar, the better prepared will he be to identify macroscopically a given collection of larvae.

In a tabular analysis of diagnostic macroscopic characters of the larvae of the more common *Anopheles* species of Assam, Manson (1934) uses general body coloration, general body size, color and size of head, pattern of spotted markings on dorsum, and degree of serration of abdominal outline.

No mutually exclusive characteristics have been found which will serve to distinguish macroscopically *all* of the larvae of any of the common Georgia species of *Anopheles* from *all* the larvae of any other species. However, some specimens of each species exhibit characteristics which

are considered diagnostic, and on a combination of characteristics, many specimens can be given a macroscopic identification sufficiently reliable to leave little doubt as to its correctness.

In some cases it has been rather difficult to decide just what macroscopic characteristics have been employed in the separation of larvae of different species when the macroscopically visible differences between

TABLE 2.—*Table of macroscopic characteristics of 4th-instar* Anopheles *larvae*

	A. quadrimaculatus	*A. punctipennis*	*A. crucians*
General coloration	Majority yellow, green, or greenish; many dark brown or black. Head of most light-colored specimens orange red, paler posteriorly (such specimens usually have broad light area involving anterior margin of thorax thus continuing posterior "paling out" of head coloration.	Majority dark brown or black; some light green; (specimens from shallow, turbid puddles usually grayish). Light specimens with reddish head not uncommon but head of these rarely lighter posteriorly or bordered by light coloration on anterior margin of thorax.	Majority dusky brown, dark brown, or dark green. Some dull green specimens with reddish head but head of these not lighter posteriorly and rarely if ever bordered by white area on anterior margin of thorax.
Presence of dorsal linear white markings	Often present, especially on yellowish and dark specimens.	Sometimes present; grayish specimens from turbid puddles almost all with some dorsal pattern.	Often present but to minimum extent on most specimens.
Pattern of white or light dorsal linear markings	Arranged as a central linear band or segments of such a band. In many specimens band widens anteriorly on thorax to form broad triangle with base along anterior thoracic margin. At abdominal segments 3 and 5 light color concentrations diamond shaped. (Rarely, light spotting on a specimen limited to a pair of whitish marks on abdominal segments 3 or 5—although a dark area may separate these marks, they do not lie parallel but can be seen to represent the anterior margins of a more or less obscured "diamond spot").	Arranged as a central linear band or segments of such a band. Whitish coloration widening anteriorly on thorax to form broad triangle in very few specimens. Light color concentrations at abdominal segments 3 and 5 diamond shaped (bicollorous "diamond spots" characteristic of grayish specimens from turbid puddles).	Characteristically arranged in two, usually thin, parallel dorso-linear stripes or paired segments of the stripes. Whether white coloration consists of continuous lines, several paired segments of the lines, or a mere pair of light parallel dashes on abdominal segment 3 or 5, a narrow, central linear dark area separates the coloration (in almost all specimens) into bilaterally symmetrical halves. (Any white coloration not centrally divided consists of segments squared at the ends—never "diamond shaped.")
Activity	Variously lively or lethargic in feeding and swimming at water surface, but rarely "nervous."	Characteristically "nervous," exhibit rapid feeding motions, and move quickly and frequently from one place to another (particularly when confined in a container).	Characteristically lethargic not lively in swimming along water surface or in feeding motions.
"S"-shaped flexure at water surface	Majority of specimens, while at water surface, will contract body into "S" shape—especially if container in which they are placed is gently agitated. ("S"-shaped flexure usually relaxed after about one minute.)	Many specimens, while at water surface, will contract body into "S" shape—especially if container in which they are place is gently agitated.	Specimens never exhibit "S"-shaped flexure (occasionally specimens raking mouth brushes on comb of eighth abdominal segment or turning head under abdomen nearly simulate "S"-shaped flexure).

TABLE 2.—(*Continued*)

	A. quadrimaculatus	*A. punctipennis*	*A. crucians*
Relation to surface film	Specimens may be dislodged from contact with surface film by sharp blows on container in which they are placed. Most dislodged specimens apply mouth parts to spiracular plate while sinking downward of own body weight; some swim downward voluntarily; others return more or less abruptly to surface.	Specimens are dislodged from contact with surface film with much difficulty, however, frightened specimens usually release contact with surface film and swim to bottom voluntarily —and often abruptly back to surface without resting at bottom (specimens which lose contact with surface film will rarely sink to bottom of own body weight).	Specimens are easily dislodged from contact with surface film by sharp blows on container in which they are placed. Usually dislodged specimens assume a rigor with anterior and posterior ends bowed dorsally to form a rough arc or crescent and in this condition sink downward of their own body weight. Specimens only rarely swim forcibly to bottom when disturbed in a container.
Head outline and angle of antennae	Head appears large and square; antennae apparently make at least 45-degree angle with longitudinal axis.	Head appears small and rounded; antennae usually not conspicuous.	Head appears rather "egg-shaped," elongate, and broader posteriorly than anteriorly. Antennae apparently make less than 45 degree angle with longitudinal axis.
Thoracic outline	Thoracic outline (especially "shoulders") appears squared.	Thoracic outline (especially "shoulders") of most specimens appears rounded.	Thoracic outline appears oblong without markedly abrupt right angles at "shoulders."
Appearance of abdomen (viewed in dorsal aspect)	Margins of abdomen appear quite serrate.	Margins of abdomen appear moderately serrate.	Margins of abdomen appear as series of evenly rounded humps.
Size and proportions	Many specimens large and robust.	Many specimens rather small and delicate.	Most specimens of average size; larger specimens appear elongate and loosely constructed.

them have appeared to be poorly defined. As differences that may seem to be of a rather trivial nature have been employed in some such cases and in a supplementary capacity quite often, these differences have been included in the Table of Macroscopic Characteristics. Characteristics are given in the Table as typical of *most* larvae of a given species when some larvae of that species have been observed which have not exhibited these characteristics; in fact, certain specimens possess some attributes that are considered typical of larvae of one species and other attributes that are characteristic of larvae of another—these are the larvae that in practice have been considered "difficult," and identification of these cannot be successfully effected without resort to the microscope unless more reliably diagnostic characteristics can be discovered. The mistakes that have been made in the macroscopic identifications (as shown in Table 1) have probably resulted in part from an occasional hasty or careless application of the macroscopic criteria to specimens to be identified but in other cases represent exceptional specimens of one species which exhibited a preponderance of the characteristics which are considered to be indicative of another species. In general, the larvae of *Anopheles crucians* and *A.*

georgianus seem to have less in common with larvae of *A. quadrimaculatus, A. punctipennis,* and *A. walkeri* than do these latter with each other.

The reference to color in the Table of Characteristics as of some diagnostic assistance is based on the rough proportions of larvae of each species that have been observed in the various listed colors or shades of color since each color has been found represented by at least a few larvae of each species. The nature of the dorsal white-spotted coloration of specimens thus spotted is of more diagnostic value than general body color, especially for separating larvae of *A. crucians* from those of the other species. Boyd (1930, p. 203) notes that this spotting on anopheline larvae has been considered diagnostic by some, and Manson (1934) finds these markings of assistance in distinguishing larvae of Indian *Anopheles* in Assam. Some larval specimens of each species exhibit this pattern of central dorso-linear white-spotting which may either be in the form of a continuous stripe (or paired stripes) or in the form of one or more segments or portions of the stripe. When limited to segments of the stripe, these will be at abdominal segment 3, abdominal segment 5, or on the thorax, and in specimens which have the complete stripe, any concentrations or broad areas in the stripe will be at one or more of these points.

MACROSCOPIC CHARACTERISTICS OF 4TH-INSTAR LARVAE OF THE LESS COMMON SPECIES

Too few specimens of the larvae of *A. walkeri* and *A. georgianus* have been observed since the significance of macroscopic appearance was recognized to allow for a very complete analysis of the various macroscopic characteristics of these larvae. In general, the larvae of *A. walkeri* appear more similar to larvae of *A. quadrimaculatus* than to those of the other species. The dorsal white coloration on the spotted specimens consists, as in *A. quadrimaculatus* and *A. punctipennis,* of a central linear band or portions of the band, but on many specimens has been noted to branch anteriorly on the thorax to form a narrow-armed "Y." At the water surface, specimens often contract into an "S" shape as do *A. quadrimaculatus* and *A. punctipennis.* In shape of head and body outline and proportions, most specimens appear similar to larvae of *A. crucians* although some specimens appear more similar to larvae of *A. quadrimaculatus* in these respects.

Larvae of *A. georgianus* appear generally similar to larvae of *A. crucians* although those of the former species seem to exhibit less white coloration dorsally and to be more inclined to swim voluntarily to the bottom when disturbed.

SUMMARY

Following a period of relatively intense study in Georgia of the macroscopic appearance of fourth-instar larvae of *Anopheles quadrimacu-*

latus, A. crucians, and to a lesser extent of *A. punctipennis,* Georgia specimens of these species were distinguished without magnification with a rather high degree of accuracy. A small percentage of specimens were found to present an appearance which made decisions as to their identity difficult. After two years of experience with the macroscopic characteristics of the larvae of Georgia anophelines, an error of nearly 4% was made in the attempted macroscopic identification of over a thousand larvae consisting of a mixture of nearly equal numbers of specimens of the three common species. Third-instar specimens were distinguished with a slightly higher percentage of error.

The fact that no table of mutually exclusive macroscopic characteristics has as yet been prepared which will serve satisfactorily to separate the larvae of the three species and that an attempted analysis of the characteristics employed in such a separation is largely based on relative appearance, indicates that (for the present at least) identification of the larvae by eye cannot be made a reliable substitute for the commonly employed identification by microscope that is based on the larval chaetotaxy. However, an ability to recognize the majority of larvae of the several species when encountered in the field can be achieved with practice, and may be employed to much advantage in studies of the associations of the larvae of each species with various aquatic "microhabitats." Thus when larvae are collected from a variety of different vegetations and other habitats in a single pond, subsequent discovery by microscopic determinations alone that three species are represented in the collection will not reveal much as to the exact situations from which specimens of the different species were taken; while a macroscopic field recognition of these larvae as the collecting proceeded would reveal the species of the larvae present in each type of vegetation or other habitats and the relative proportions in which they occur in each; subsequent determinations by microscope would check the identifications made in the field. Also, it is often a distinct advantage in practical survey work to know while in the field, and with a fair degree of certainty, of just what species a given collection of larvae may consist.

Bibliography

Bellamy, R. E. 1939 An anopheline from inland Georgia resembling the brackish-water race of *Anopheles crucians.* J. Parasitol. **25**: 186.

Boyd, Mark F. 1930 An Introduction to Malariology. Cambridge, Mass. 437 pp.

Bradley, G. H. 1936 On the identification of the mosquito larvae of the genus *Anopheles* occurring in the United States. South. Med. J. **29**: 859–861.

King, W. V. 1939 Varieties of *Anopheles crucians* Wied. Am. J. Trop. Med. **19**: 461–471.

King, W. V. and G. H. Bradley 1941 General morphology of *Anopheles* and classification of the Nearctic species; pp. 63–70 in "A Symposium on Human Malaria," Washington, D. C. 398 pp.

Manson, D. 1934 Some notes on the identification of some anopheline larvae by macroscopic methods. Rec. Malar. Surv. India, **4**: 197–203.

A PARASITE OF THE MACRONUCLEUS OF *VORTICELLA*

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In a sample of water from each of two water troughs used by horses at the Frances Simes Hastings Natural History Reservation, Monterey County, California, many specimens of *Vorticella* were found with the macronucleus parasitized by bacteria. By comparison with the taxonomic accounts by Noland and Finley (1931) and Kahl (1935) the species of ciliate seemed to be *V. similis* Stokes. I am indebted to Dr. J. M. Linsdale, Director of the Reservation, for the collections of water containing the ciliates. The names of the troughs in the following paragraph are those used by him in the geographical records of the Reservation.

The first sample was obtained from Upper Barn Trough on February 5, 1941. Upon examination of floated coverglasses on the following day I found numerous specimens of *Vorticella,* and all of them had parasitized macronuclei. Those observed on the next day also were all parasitized, but the number had greatly decreased. The ciliate was present in a collection made from the same trough on February 13, but no nuclear parasites were found. The second sample with parasitized *Vorticella* was obtained from Cow Pasture Trough on March 29, 1941; in this material there were also many unparasitized specimens.

In the living ciliates, the hypertrophied nuclei provided an indication of the presence of the parasites, which on close examination could easily be seen as small refractile rods (Fig. 3). One specimen with a parasitized nucleus was seen in fission, with the macronucleus in a dumb-bell form. Fission was completed while the specimen was watched. Detailed studies of the parasite were made in Schaudinn-fixed, iron haematoxylin-stained preparations.

When not parasitized, the greatly elongated macronucleus has a varying width that often is about 4–5 μ. Nuclei only a little thickened, and with few scattered parasites, were seen. In many instances the parasitized macronucleus had a width of 10, 13, or more microns, usually retaining its elongated form. At an extreme of hypertrophy, it had become a large ellipsoidal body measuring about $\frac{2}{3}$ the length of the ciliate and $\frac{3}{4}$ of its width. In one specimen the parasitized macronucleus had fragmented into two rounded bodies, one with a diameter of 23 μ and the other of 15 μ.

There is a marked alteration of the substance of the macronucleus even when the parasites are present in small numbers. Nuclei not parasitized contain numerous close-set, deep-staining, relatively large granules

Received for publication, October 8, 1941.

of chromatin (Fig. 1). In parasitized nuclei on the same slides the macronuclear substance is gray, and its texture at moderate magnification seems about homogeneous. At the highest magnification it may be seen to be alveolar or obscurely granular, but there is no sharp resolution of structure like that of the normal nucleus.

The parasites are deep-staining rods, straight or slightly curved in

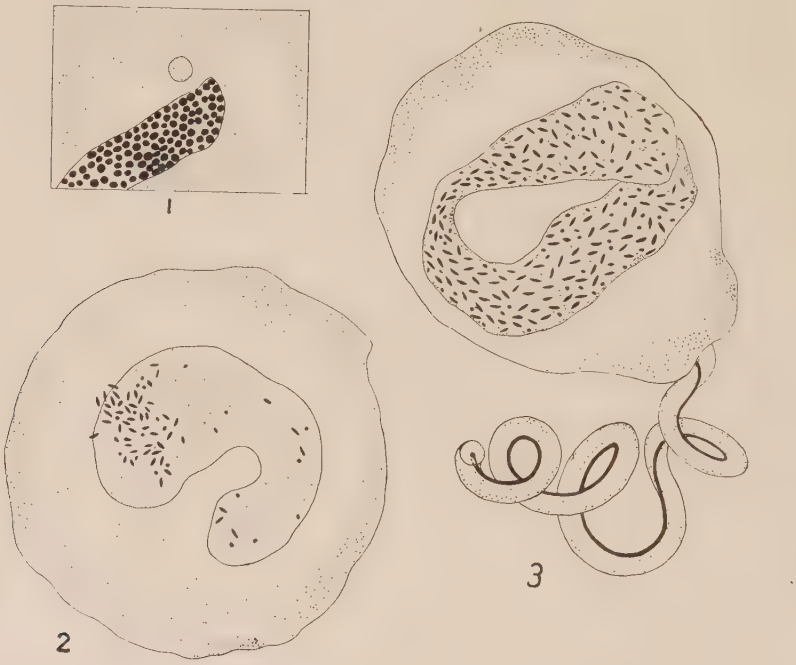

FIG. 1. A portion of the normal macronucleus of *Vorticella,* showing deep-stained chromatin granules.

FIG. 2. Macronucleus of *Vorticella* parasitized by bacteria, and hypertrophied most in the region where there are most parasites. A few bacteria of the same form are in the cytoplasm just outside the macronucleus.

FIG. 3. Macronucleus heavily parasitized with evenly distributed bacteria. All the parasites are fusiform; those that appear as granules are represented as seen from the end.

All figures × 1275. Fixation Schaudinn. Stain Heidenhain's iron haematoxylin.

In preparation of these figures, assistance rendered by personnel of Work Projects Administration, Official Project 65-1-08-113, Unit C 1, is acknowledged.

form, and homogeneous in so far as demonstrated (Fig. 3). Their length ranges from about 0.5 μ to 1.5 μ. Some of the rods have pointed ends and a neat spindle form. Others are rounded at one or both ends. Each rod lies in the substance of the macronucleus in a vaguely defined clear area. They are oriented in all directions, and in most instances observed were uniformly distributed throughout the macronucleus. In several specimens the parasites were for the most part grouped in one region of

the nucleus. This part of the nucleus was more greatly enlarged than the part with few parasites. Occasionally a few parasites were found to lie outside the macronucleus, but in contact with it (Fig. 2).

Numerous vorticellas with parasitized nuclei were studied, and in none were there rods of larger size than those described above.

Parasites of the macronucleus of ciliates have been recorded by many authors. The most frequently reported parasite is of the *Holospora* type. This organism was observed in *Paramecium caudatum* by Müller (1856), Balbiani (1861), and many others. (See review by Kirby, 1941.) Recently it has been studied by Wichterman (1940). It may reach a length surpassing 20 μ; Fiveiskaja (1929) reported a maximum of 30 μ. There are stages in development that are spindle-shaped and only 3–6 μ long, but these develop rapidly into the larger forms (Fiveiskaja). Stein (1867) reported threads much like those in the nucleus of *Paramecium* in *Pleuronema chrysalis* and *Enchelyodon farctus.*

Parasites of smaller size were found in macronuclei of *Chilodon cucullulus* (4 μ long, according to Engelmann, 1862); *Blepharisma lateritia* (Engelmann, 1862); *Stentor roeselii* (3.7–5.6 μ, Stein, 1861, 1867); and *S. polymorphus* (6 μ, Balbiani, 1893). These parasites had in common differentiation of at least some forms for part of the length into a denser, more refractile substance, with the remainder a clearer substance. This differentiation is characteristic of *Holospora.* Engelmann (1876) reported in *Stylonychia mytilus* enlarged bodies that seemingly were nuclei or nuclear fragments, which were densely infested with cylindrical or biscuit-formed bacteria at most 3 μ long, ranging down to ½ this size. The same bacteria, he stated, were abundant in the water.

I have found no record of parasites of the nuclei of species of the genus *Vorticella.* In the taxonomic accounts by Noland and Finlay (1931) and Kahl (1935) nuclear parasites are not mentioned. The authors must have observed a great deal of material, and their failure to record the parasites must indicate that they are not a common occurrence. For other peritrichs, there seems to be only the report by Greeff (1871) of rods in the macronucleus of *Epistylis flavicans.* He recognized that these structures were similar to those previously described in *Paramecium.*

A unique macronuclear parasite was found in *Prorodon* by Stein (1867), Švec (1897), and Kahl (1930). Stein noticed in the unenlarged macronucleus of *Prorodon teres* spindle-shaped bodies parallel to one another and attaining 36 μ in length. Švec named a new species, *P. nucleatus,* the macronucleus of which contained 4 or 5 large rods. Kahl stated that similar rods are found at times in various species of *Prorodon,* and must be regarded as parasites.

So far as I have been able to learn, the minute bacteria that I have observed in the macronucleus of the species of *Vorticella* are not the same as any nuclear parasite hitherto described. The parasite causes structural alteration and hypertrophy of the macronucleus.

Bibliography

Balbiani, E.-G. 1861 Recherches sur les phenomenes sexuels des infusoires. J. Physiol. Homme et Animaux **4**: 102–130; 194–220; 431–448; 465–520.

———1893 Nouvelles recherches expérimentales sur la mérotomie des infusoires ciliés. Ann. Microg. **5**: 1–25.

Engelmann, T. W. 1862 Zur Naturgeschichte der Infusionsthiere. Z. Wissensch. Zool. **11**: 347–393.

———1876 Ueber Entwickelung und Fortpflanzung von Infusorien. Morphol. Jahrb. **1**: 573–635.

Fiveiskaja, A. 1929 Einfluss der Kernparasiten der Infusorien auf den Stoffwechsel. Arch. Protistenk. **65**: 275–298.

Greeff, R. 1870, 1871 Untersuchungen über den Bau und die Naturgeschichte der Vorticellen. Arch. Naturg. 36 Jahrg. **1**: 353–384. 37 Jahrg. **1**: 185–221.

Kahl, A. 1930 Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata. In Dahl: "Die Tierwelt Deutschlands," Jena. 18. Teil.

———1935 Idem 4. Peritricha und Chonotricha, Ibid. 30. Teil.

Kirby, H. 1941 Organisms living on and in protozoa. In Calkins and Summers: "Protozoa in Biological Research," Columbia Univ. Press, New York.

Müller, J. 1856 Einige Beobachtungen an Infusorien. Monatsb. K. Preuss. Akad. Wissensch. **1856**: 389–393.

Noland, L. E. and Finley, H. E. 1931 Studies on the taxonomy of the genus *Vorticella*. Tr. Am. Micr. Soc. **50**: 81–123.

Stein, F. (Ritter) von 1861 Über die Conjugation der Infusionsthiere und über die geschlechtliche Fortpflanzung der Stentoren. Sitzungsb. K. Böhm. Gesellsch. Wissensch. **1861**: 62–77.

———1867 Der Organismus der Infusionsthiere. II. Abth. (1) Darstellung der neusten Forschungsergebnisse über Bau, Fortpflanzung und Entwickelung der Infusionsthiere. (2) Naturgeschichte der heterotrichen Infusorien. Leipzig.

Švec, F. 1897 Beiträge zur Kenntnis der Infusorien Böhmens. I. Die ciliaten Infusorien des Unterpočernitzer Teiches. Česka Akad. Fr. Jos. Prag. (Math-Nat.) **4**: 29–47.

Wichterman, R. 1940 Parasitism in *Paramecium caudatum*. J. Parasitol. **26** Suppl.: 29.

FURTHER STUDIES UPON THE CHEMISTRY OF *MACRACANTHORHYNCHUS HIRUDINACEUS*

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In two previous papers (von Brand, 1939, 1940) some aspects of the chemical composition of *Macracanthorhynchus hirudinaceus* were discussed. The present study is an extension of these investigations and deals with the polysaccharides and some inorganic constituents occurring in the worms.

THE POLYSACCHARIDES

As stated previously (von Brand, 1939) a polysaccharide can be isolated from these parasites in fairly large quantities by Pflueger's procedure. It gives typical glycogen reactions (opalescence of the aqueous solution, deep brown-red color with iodine, sugar identified as glucose after hydrolysis). However, upon digestion with filtered saliva, a small indigestible fraction was found which gave no color reaction with iodine and reduced Fehling's solution only after hydrolysis with hydrochloric acid. Since it was clearly not glycogen, the only polysaccharide so far described from any parasitic worm, it seemed of interest to extend these observations.

The crude polysaccharide was isolated from about 500 gm of freshly collected worms by Pflueger's method. It was further purified by heating for two hours on a boiling water bath with 60% KOH, following which it was precipitated with alcohol once from alkaline, once, after filtration, from acid and four times from neutral aqueous solution. It was then washed repeatedly with 96% alcohol, 100% alcohol, and ether, and the whole purification process repeated once more. The yield was 4.5 gm of a pure white powder, readily soluble in water with strong opalescence. It was nitrogen-free (no Prussian blue precipitate was obtained on treatment of the filtrate of the sodium fusion of the polysaccharide with ferrous sulfate, ferric chloride and hydrochloric acid), and it was practically ash-free.

Of this substance, 200 mg were dissolved in 25 ml of distilled water and the optical rotation determined in a 1-dm tube.

$25°$ C; Na light (D)
Zero reading $= -0.07°$
Optical rotation $= +1.43° - (-0.07°) = +1.50°$

$$\alpha \frac{20}{D} = \frac{150° \times 25}{0.2} = +187.5°$$

Received for publication, October 8, 1941.

This is somewhat less than the most reliable figure for glycogen ($\alpha\frac{20}{D}=+196.6°$, according to Gatin-Gruzewska, 1904).

The material was then digested for 24 hours with filtered saliva at 37° C, bacterial growth being prevented by addition of a few drops of toluene to the solution. The indigestible fraction was isolated by precipitation with alcohol. After reprecipitation it was heated for two hours on a boiling water bath with 60% KOH, precipitated with alcohol one time each from alkaline, acid and neutralized aqueous solution. It was then digested again, and the purification process outlined above was repeated.

A small amount of white material resulted which gave an opalescent aqueous solution. It was hydrolyzed with dilute hydrochloric acid. To a drop of the hydrolyzate a little phloroglucinol was added; upon heating on a boiling water bath, a red color developed. This indicated the presence of either galactose, pentose or glycuronic acid. The rest of the hydrolyzed material was heated for two hours on a boiling water bath with 1 ml of concentrated nitric acid. Upon standing over night a water-white crystalline material was obtained which showed the crystalline form of mucic acid (2,3,4,5-tetrahydroxyhexanedioic acid) under the microscope, but unfortunately the amounts were not sufficient for a satisfactory melting point. It seems very likely, however, that we were dealing with galactose, since neither pentose nor glycuronic acid would yield a precipitate upon heating with nitric acid.

The question whether the saliva-resistant fraction is galactogen or a galactose-containing polysaccharide sui generis cannot be answered definitely at the present time. The lowered value of the optical rotation of the original polysaccharide mixture as against pure glycogen would well agree with the presence of galactogen which shows in pure state an optical rotation of $\alpha\frac{20}{D}=-22.7°$ (May, 1932). This latter polysaccharide has been found so far in various molluscs and in vertebrates (May, 1934). Its presence in a representative of a third phylum would not be surprising. Basing the calculation on the available figures for optical rotation, it might be assumed that the original polysaccharide mixture contained about 4% galactogen, corresponding roughly to 0.04% of the fresh weight of the worms. For a complete satisfactory analysis therefore about 10 kg or about 2000 adult females would be required, an amount difficult to collect.

INORGANIC SUBSTANCES

In view of the growing realization of the importance of the "trace elements" in many biological fields, it was decided to subject the worms to a spectrographic analysis. This seemed the more important as very

little is known about the inorganic constituents of any parasite. The most complete analyses are those of Flury (1912) on ascarids and those of Salisbury and Anderson (1939) on *Cysticercus fasciolaris*. The former found the metals Na, K, Ca, Mg, Fe and Al, whereas he was unable to demonstrate the presence of Cu and Mn. The latter mention as metals Na, K, Ca, Mg and Fe. In *Macracanthorhynchus* the presence of only the following metals has been shown in a qualitative way: Na, K, Ca, Mg and Fe (von Brand, 1939).

The worms intended for the spectrographic analysis were collected especially for this purpose, care being taken to avoid any contamination with metals (except, of course, Na, since the worms had to be washed in isotonic NaCl solution after isolation). They were dried, ground in a mortar and analyzed qualitatively with a quartz Littrow spectrograph.

Four different samples were analyzed: females; males; eggs and ovaries; body fluid of females after centrifuging off the eggs and ovaries. The following metals were found in all four samples: Na, Ca, Mg, Mn, Al, Fe and Cu. The range of wave length in which the characteristic lines of the elements were located was in the near ultra-violet. In this range potassium lines are very faint and their presence was not demonstrated. However, potassium lines appeared clearly in the far ultra-violet. In addition to the metals, the spectrograph revealed the presence of P and Si.

In view of the importance that Fe and Cu play in the constitution of respiratory pigments occurring in invertebrates (haemoglobin, haemocyanine), quantitative determinations of these substances were performed; although nothing is known at the present time about the presence or absence of any respiratory pigment in acanthocephalids. The sample used for these determinations consisted of dried females, containing 3.80% ash.

Iron was determined according to the method of Linzel (1933).

2.455 mg gave 7.2×10^{-3} mg Fe
2.775 mg gave 8.4×10^{-3} mg Fe
Found: Fe = 0.29, 0.30%

Copper was determined according to the method of Eisler, Rosdahl and Theorell (1936).

2.133 mg gave 0.000 mg Cu
9.998 mg gave 0.000 mg Cu

With this method Cu cannot be demonstrated if present in amounts less than 0.01%. Since all the spectrographic examinations were positive for Cu, it is legitimate to assume that its concentration was less than 0.01%.

SUMMARY

1. *Macracanthorhynchus hirudinaceus* contains besides true glycogen small amounts of a polysaccharide resembling and perhaps identical with galactogen.

2. Spectrographic analysis revealed the presence of the following metals in the worms: Na, K, Ca, Mg, Mn, Al, Fe and Cu.

BIBLIOGRAPHY

BRAND, TH. VON 1939 Chemical and morphological observations upon the composition of *Macracanthorhynchus hirudinaceus* (Acanthocephala). J. Parasitol. **25**: 329–342.

————1940 Further observations upon the composition of Acanthocephala. J. Parasitol. **26**: 301–307.

EISLER, B., K. G. ROSDAHL AND H. THEORELL 1936 Ueber die Mikrobestimmung des Kupfers mit Hilfe der lichtelektrischen Photometrie. Biochem. Z. **285**: 76–77.

FLURY, F. 1912 Zur Chemie und Toxikologie der Ascariden. Arch. Exper. Path. u. Pharm. **67**: 275–392.

GATIN-GRUZEWSKA, Z. 1904 Das reine Glykogen. Pflueger's Arch. **102**: 569–591.

LINTZEL, W. 1933 Zur Methodik der Mikrobestimmung des Eisens in biologischem Material. Z. Exper. Med. **86**: 269–274.

MAY, F. 1932 Ueber den Galaktogengehalt der Eier von *Helix pomatia*. Z. Biol. **92**: 325–330.

————1934 Chemische und biologische Untersuchungen ueber Galaktogen. Biologischer Teil: Galaktogen Nachweis bei verschiedenen Tierklassen und beim Menschen. Z. Biol. **95**: 614–634.

SALISBURY, F. L. AND R. J. ANDERSON 1939 Concerning the chemical composition of *Cysticercus fasciolaris*. J. Biol. Chem. **129**: 505–517.

CERTAIN NUTRITIONAL REQUIREMENTS OF THE FOWL CESTODE *RAILLIETINA CESTICILLUS* (MOLIN) AS DEMONSTRATED BY SHORT PERIODS OF STARVATION OF THE HOST[1,2]

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Because of the lack of a digestive system in cestodes, the nutrition and the nutritional habits of tapeworms have long interested parasitologists. However, due primarily to the difficulties involved in studying the processes which normally occur only within the intestine of the host, comparatively little is known about the food requirements of this unique group. All attempts to simulate normal conditions of warm-blooded animals by the use of in vitro methods have met with disappointment as mere survival of tapeworms for not more than a few days is all that has thus far been accomplished. Nevertheless, most of the knowledge of the physiological processes of tapeworms has come from the in vitro experiments of such workers as von Brand (1933) and Wardle (1937a). In this type of work portions of *Moniezia expansa* were subjected to various treatments for a few hours, analyzed chemically, and their composition compared with that of untreated portions of the worm which had been previously analyzed.

Another type of experimental study has been made by Levine (1938) and more recently by Hager (1941) in which the diet of the host was altered in various ways and the effects upon proglottid production or onchosphere count were measured. It appears that only von Brand has combined the two methods by a study of chemical composition of worms after the host has been starved or given special diets.

Recent observations upon short starvation periods of chickens infected with *Raillietina cesticillus* (Molin) led to the conclusion that rather profound changes must be induced in these parasites by lack of proper food in the gut of the host (Reid, 1940). Therefore, a more comprehensive study was made by using the combination of starvation and chemical analyses of worms removed from starved and from control hosts.

MATERIAL AND METHODS

Both chickens and beetles (intermediate hosts used in producing experimental infections of the fowls) were reared according to the technique

Received for publication, October 27, 1941.

[1] Contribution No. 229 from the Department of Zoology, Kansas Agricultural Experiment Station, Manhattan, Kansas, and the Department of Biology, Monmouth College, Monmouth, Illinois.

[2] A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The assistance of Dr. James E. Ackert under whose direction the work was carried out is gratefully acknowledged.

of Reid, Ackert, and Case (1938) with certain modifications and improvements (Reid, 1942). Worms to be analyzed were removed immediately after the fowl had been killed by deeply scraping the mucosa in the region under the scolex. In many cases this served to free the deeply embedded scolex, but because speed was essential, and the weight involved negligible, the recovery of the scolex was not considered essential. The worms were quickly washed in mammalian Ringer's solution to remove any debris or mucus adhering to them and rolled on a dry piece of filter paper to remove surface moisture.

Worms to be analyzed for glycogen were placed immediately in 0.5 cc of 30 per cent KOH and the glycogen determinations made by the micromethod of Heatley (1935). Acid hydrolysis of the glycogen was carried out with a 2 N solution of sulfuric acid instead of the usual 2.2 per cent hydrochloric acid since Sahyun (1931) has shown that the chlorine ion interferes with the development of the final blue color when the Folin and Wu macro sugar method (1920) is used, while the sulfate ion does not so interfere.[3]

Worms to be used in fat determinations were removed in a similar manner, but larger numbers of worms were included in each sample. Immediately upon removal, the wet weight of the worms was obtained by weighing in small test tubes. The worms and the water that had oozed out in the weighing tube were placed on weighed, ash-free, fat-free filter paper and permitted to dry at room temperature. Last traces of moisture were removed in vacuo over calcium chloride. The ether-soluble portion was extracted by means of a Bailey-Walker fat extraction assembly. After weighing back the filter paper containing the residual material, Kjeldahl determinations for organic nitrogen were made on some of the samples while other samples were ashed in a muffle and the ash content determined.

REVIEW OF LITERATURE

NUTRITION

Because of the economic importance of the nematodes, the nutritional habits of this group have been more extensively studied than those of other helminths. Lapage (1937) in his comprehensive review of the nematodes included a section on nutrition and its effects on the host. Ackert and Whitlock (1941) have reviewed the feeding habits of the nematodes. McCoy (1935) reviewed the physiology of the helminth

[3] Although it is customary to multiply the reducing sugar value by Pflüger's correction factor of 0.927 to obtain true glycogen values, this figure has recently been questioned. Kerly (1930) used 0.957 while Wardle (1937a), calculating from results obtained by Bell and Young, used 0.961. Following the example of some biochemists in waiting for the question to be settled, no correction of the reducing sugar values has been made in the present studies.

parasites including a section on nutrition. Histological examination of the gut and its contents as well as numerous ingenious experimental procedures have shown that there is wide variation in the food and feeding habits of the nematodes. These differences are generally correlated with the position of the parasite within the host. Some are saprozoic, living on digested food of the host; others live on bacteria; while others feed on tissue, tissue exudate or body fluids. Although equipped with a digestive system, some forms such as the filaria probably absorb nutriment through the body wall.

Studies on fluke nutrition are less extensive, but there appear to be similarities with the modes of nutrition of the nematodes. Hsü (1938) in a study of both nematode and trematode tissue-parasites found a higher correlation between the location of the parasite and its food than between food and the phylum to which the parasite belonged. Thus *Paragonimus ringeri* and *Spirocerca sanguinolenta* which live in nodules, feed on cells passing through the wall of the nodule, while *Schistosoma japonicum, Dirofilaria immitis,* and *Rhabdias* sp. which live in the vascular system, take only cells present in the general circulation.

Entirely different methods must be applied to the study of the nutrition of cestodes since no digestive system is present; the food must therefore consist of easily diffusible substances present in the gut of the host. Most of the evidence available on the nutrition of this group has come from physiological studies, a review of which was made by Wardle (1937b).

It is now well established that cestodes, as well as other intestinal helminths, contain large quantities of glycogen and fat. According to von Brand (1933), glycogen plays the major role in the metabolism of this group, while fat is at least partially a waste product. He recorded slight decreases in glycogen content in sections of *Moniezia expansa* after six hours of in vitro starvation. In similar experiments in which glucose was added to the media, no significant change in the glycogen content was noted.

Wardle (1937a) pointed out that although the polysaccharide content of the worms is generally conceded to be synthesized from glucose, there is little experimental evidence to support this view. Wardle's experiments, likewise with *M. expansa* during six hours of in vitro starvation, suggest that glycogen is consumed in quintuple Tyrode, one per cent sodium glycocholate solution, and in bile saline. He pointed out that although these results are suggestive of polysaccharide loss due to increased function of the musculature in these solutions, the possibility of glycogen loss by exosmosis must be considered. These results would be more convincing were sufficient chemical analyses available so that statistical methods could be used as the criteria of significance, in place of

the extreme range in variation of four controls. By similar methods Wardle reported gains in polysaccharide content when other sugars were substituted for glucose.

More recently Markov (1939), who has been able to maintain worms under in vitro conditions for longer periods of time, has demonstrated that glucose is absorbed by the cestodes *Ligula intestinalis, Eubothrium rugosum* and *Diphyllobothrium latum,* and the nematode *Triaenophorus nodulosus,* and that the rate of glycogen consumption is independent of the initial glycogen content.

Another type of evidence for the need of carbohydrate food in cestodes was presented by Levine (1938). Indigestible substances such as bran or cellophane, when added to the host diet in sufficient quantity to provide the necessary bulk but also to greatly reduce the carbohydrate content, caused a marked reduction in proglottid production in *Davainea proglottina.*

Experiments with other factors in the diet of the host upon cestodes have been conducted by Hager (1941) and Alicata (1939, 1940). Hager showed that a factor, associated with the vitamin G complex, was necessary for normal onchosphere production by the rat tapeworm *Hymenolepis diminuta.* Alicata found indications that a diet high in animal-protein permitted fewer worms to develop than a diet high in plant-protein in experiments with *Hymenolepis exigua* of the fowl. The dietary effects produced in this latter experiment confirm similar findings with the nematode *Ascaridia lineata* by Ackert and Beach (1933).

STARVATION

The effects of starvation of the host upon the intestinal fauna seem to have had little systematic study, but the literature contains several interesting observations on the subject and starvation has been used as an experimental tool in isolated cases.

In the trematodes Beaver[4] observed that "for *Echinostomum revolutum* the general well being of the host, including nutrition, directly influenced the parasite. Sick or starved hosts lost their worms." Burlingame and Chandler (1941) noted that fasting rats lost the acanthocephalan worms *Moniliformis dubius,* 65 out of an estimated 227 worms in 12 rats having been lost in five days.

In studies on the food of the fowl nematode *Ascaridia lineata,* Ackert, Whitlock and Freeman (1940) showed that worms in chickens given only water per os grew very little during the first eight days of starvation and not at all between eight and 27 days. Although both control and experimental chickens lost worms during the course of the starvation period, the experimental chickens lost them at a much faster rate than did the controls.

[4] Personal correspondence, April 21, 1941.

With the cestode *Davainea proglottina,* Levine (1938) found that complete starvation of the host for 24 hours caused a decrease in proglottid production during the following week.

In conjunction with the present work, a detailed study (Reid, 1940, 1942) of the effects of starvation on *Raillietina cesticillus* revealed that starvation of hosts infected with this worm for 24 to 48 hours resulted in the loss of the strobila of all of the worms present in the gut of the host. The scolex, however, was unaffected by starvation periods which extended as long as 20 days, and was able to regenerate a new strobila upon resumption of normal feeding habits in the host. Furthermore, newly established cysticercoids, although sometimes able to maintain their respective positions in an empty gut, did not grow appreciably until the fowl was placed upon normal feed.

EXPERIMENTAL DATA

CARBOHYDRATE CONTENT OF WORMS BEFORE AND AFTER SHORT PERIODS OF HOST STARVATION

Chemical determination of possible food reserves in conjunction with host starvation were undertaken to determine whether this loss of strobilae in starved hosts was due to an actual starvation of the worm tissue or to some other factor in the host-parasite relationship. Since it has been established that glycogen is the predominant carbohydrate in tapeworms as well as other intestinal parasites and since it is likewise well-known that the glycogen store of higher animals is readily depleted dur-

TABLE 1.—*Glycogen level in tapeworms from a starved and an unstarved host. (Exper. 1)*

Hour killed and treatment	Chicken number	Total worms	Worms in sample	Per cent glycogen of worm wet weight
4 PM On feed until killed	A 897	10	1	4.83
			1	3.62
			1	2.52
			1	4.38
			1	4.55
			1	6.45
			1	4.85
			1	5.25
			1	4.95
				Mean 4.60
6 PM Off feed for 24 hours before killing	A 1092	17	1	0.09
			1	0.06
			1	0.10
			1	0.03
			1	0.32
			1	0.49
			1	0.47
			3	0.46
			3	0.18
				Mean 0.25
			Difference in means	4.35*
			Standard error	0.387

* Highly significant.

ing starvation, it followed that glycogen might be the logical food reserve to investigate first.

Exper. 1.—The first glycogen analyses were made upon individual worms from a chicken killed at 4 PM after it had been feeding normally, and upon worms from a chicken which had been off feed for 24 hours. The mean glycogen percentage calculated on the basis of the wet weight of the worms for nine cestodes from the unstarved chicken was 4.60 per cent (Table 1) while that on nine analyses of worms from starved hosts was 0.25 per cent. Although the glycogen level of the latter group of worms was so low that the glucose readings had to be estimated, in all cases these estimates were probably high. It is apparent, therefore, that the glycogen content of the worms in the starved host was reduced to a mere trace. Furthermore, it was of interest to note that the worms removed from this chicken were flaccid and very much less active than worms from the normally fed chicken.

Exper. 2.—While 23 tapeworms were available for study in Exper. 1, these worms were from but two chickens. Accordingly, a second experiment was planned in which worms from eight chickens were analyzed, four of the chickens being normally fed, and four having been without feed for 20 hours before the worms were removed. The starvation time in this experiment had to be reduced by four hours since repeated efforts again to obtain whole worms from chickens starved for 24 hours met with failure. Another change in procedure was the use of more worms per sample so that sufficient glucose was present for accurate determination. It may be noted (Table 2) that worms from the control chickens had a mean glycogen level, calculated on a per chicken basis, of 4.38 per cent with a range per chicken of 3.98 to 5.14 per cent. With

TABLE 2.—*Glycogen level in tapeworms from several starved and unstarved chickens. (Exper. 2)*

Hour killed and treatment	Chicken number	Total worms per chicken	Number of worms per sample	Per cent glycogen of worm wet weight	Mean per cent glycogen in worms
6–7 PM	A 1095	44	10	3.84	
On feed until killed			10	4.83	4.34
	A 1015	36	7	4.67	
			7	5.60	5.14
	A 1363	3	2	3.98	3.98
	A 1096	107	30 scoleces	4.08	4.08
				Mean	4.38
2–3 PM	A 1100	56	10	0.24	
Off feed 20 hours			10	0.51	0.38
before killing	A 1094	33	10	0.25	
			10	0.42	0.34
	A 1098	33	10	0.57	0.57
	A 1097	200	30	0.21	
			30 scoleces	0.17	0.19
				Mean	0.37
				Difference in means	4.01*
				Standard error	0.274

* Highly significant.

the starved chickens the mean per bird level was 0.37 per cent with a range between 0.19 and 0.57 per cent. The mean difference of 4.01 per cent between the two groups is highly significant.

The results of Exper. 1 and 2 show clearly that this marked fall in glycogen level is due to host starvation. Furthermore, it seems probable that this drop in glycogen content of worms in starved hosts accounts for the loss of strobilae from these worms. Ortner-Schönbach (1913) in a histological study of the distribution of glycogen in cestodes reported concentrated clumps of glycogen in the parenchyma around muscle fibers, the muscle fibers themselves being relatively free of glycogen. She presumed this glycogen to be a source of muscular energy. In the present experiments it would follow that when the glycogen supply around these muscle fibers is exhausted the muscles would no longer be able to contract normally. Using an analogy introduced by Wardle (1935), the worm is no longer able to swim effectively against the current in the intestine and is swept out by peristaltic action. The flaccid condition of worms from starved hosts would tend to support this view. Since the scolex of this species is very deeply embedded and the neck region is relatively fragile, separation usually occurs at this point.

GLYCOGEN FLUCTUATIONS DURING THE NORMAL TWENTY-FOUR HOUR CYCLE

If such a profound change in glycogen level in the worms could occur during 20 hours of host starvation which includes 12 hours of night, it seemed likely that fluctuations in glycogen content might occur during the normal 24-hour feeding cycle. Such a fluctuation would be of special interest because of the relationship between food and the proglottid production cycle. Wetzel (1932) reported such a cycle for the fowl cestode *Davainea proglottina,* a peak in proglottid production occurring at about 3: 30 PM with a gradual decrease to complete cessation during the early morning hours. In 1934, Wetzel reported similar cycles for *R. cesticillus.* The occurrence of these cycles has been confirmed by Reid, Ackert and Case (1938) for *R. cesticillus.* Levine (1938) has likewise confirmed the presence of these cycles in *Davainea proglottina.* He was able to reverse the peak to the early morning hours by changing the feeding time to the night. He thus effectively demonstrated a fact that had been suspected by other workers, namely, that this proglottid production cycle was dependent on the feeding habits of the fowl.

Exper. 3.—In order to test the possibility of a fluctuation in the glycogen level in the 24-hour cycle, worms from two groups of chickens were analyzed (Table 3). One group was killed between 6 and 7 PM, which was at the close of the normal feeding period in November, while the other group was killed between 6 and 7 AM, just before feeding had

begun. The glycogen level when calculated on a per worm basis (only samples consisting of one worm each were included in the statistical analysis) was 7.14 per cent at the evening hour while it averaged only 3.68 per cent at the early morning hour. The 12 worms analyzed from the group killed during the evening thus had an average of 3.46 per cent

TABLE 3.—*Glycogen level at the beginning and at the end of a normal feeding period. (Exper. 3)*

Hour killed and treatment	Chicken number	Total worms	Worms in sample	Location in gut cm from ampulla	Per cent of worm wet weight	Mean per cent glycogen in worms
6–7 PM	9706	2	1	8	5.72	
On feed until killed			1	14	6.17	5.94
	9703	2	1	10	8.51	
			1	10	7.85	8.23
	9716	4	1	8	8.21	
			1	10	7.75	
			1	13	8.20	
			1	13	8.29	8.11
	9709	35	1	2–8	5.31	
			1	12	6.18	
			1	13–18	6.87	
			1	18–23	6.49	
			2	30–35	6.44	
			2	41	6.34	6.30
				Means	7.14	7.13
6–7 AM	9724	33	1	8–10	4.30	
Off feed during night			1	8–10	3.81	
			1	8–10	3.54	
			1	8–10	3.66	
			1	10–16	4.69	
			1	16–20	4.38	
			1	20–25	4.41	
			1	20–25	5.38	
			1	20–25	5.15	
			1	30–38	5.06	
			2	30–38	4.36	
			19	random	4.33	4.37
	A 1122	3	1	15	3.34	3.34
	9711	9	1	8	2.14	
			1	10	3.64	
			1	18	3.68	
			1	35	3.43	
			1	35	3.77	
			1	40	3.60	
			1	45	3.01	
			1	45	3.44	3.34
				Means	3.68	3.92
				Difference in means	3.46*	3.21*
				Standard error	0.763	0.346

* Highly significant.

more glycogen than 19 worms in the morning group. As the standard error was 0.763, the difference was highly significant (Table 3). Likewise, when the glycogen level was calculated on a per chicken basis, the four-chicken group killed between 6 and 7 PM averaged 7.13 per cent while the three chickens in the 6 to 7 AM group averaged 3.92 per cent. The difference of 3.21 per cent in this case was likewise highly significant. It has been found, therefore, that upon whatever basis comparison is made, the glycogen level during the evening hours was markedly higher than that in the early morning hours.

These data are convincing evidence that the glycogen level fluctuates

during a normal 24-hour feeding cycle. It is higher in the evening after feeding has been in progress during the day, but lower during the early morning hours when no food is in the environment of the tapeworm. It seems probable that this rhythmic cycle in glycogen level during the 24-hour period is responsible for the cycle in proglottid production of the tapeworm. No explanation of this proglottid production cycle has thus far been suggested other than the correlation between it and the feeding habits of the host.

In weighing worms from different locations in the intestine for glycogen analyses, it was noted that the worms at the anterior end of the gut weighed appreciably more than those in posterior locations. In some cases those in anterior locations, although no longer than those in posterior positions, weighed twice as much. This raised the question as to whether a difference in the glycogen content due to different food conditions in the gut might be responsible for this difference in size. Study of the results indicated in Table 3, however, does not reveal any significant trends of variation in the glycogen content of worms from different parts of the gut.

Exper. 4.—To further correlate the glycogen cycle with the proglottid production cycle, analyses were undertaken at the mid-morning hour. Worms were extracted at 10 AM from four chickens which had been on feed and from five chickens which had been off feed during the morning of the experiment. The glycogen content was calculated on a per chicken basis as determined from either two or three samples consisting of two worms each. The percentages were 1.08, 1.48, 2.08, 3.00, for the chickens with feed before them continuously, while the chickens which had been off feed for 20 hours contained worms with 2.08, 1.57, 1.73, 1.90 and 1.23 per cent glycogen. Since no significant difference in the glycogen levels in these two groups was demonstrated, it must be concluded that the expected rise in glycogen content after the relatively low level of the early morning hours had not begun to take place. There is, apparently, a time lag in the building up of the glycogen store since the control birds had all begun to feed three hours before they were killed.

The data from these four experiments together with some additional data are summarized in Fig. 1. It should be noted that the first two-thirds of the curve represents the normal glycogen cycle of the cestode which is repeated from day to day, while the last third represents the glycogen levels when feed is removed the following day. The perpendicular bars represent twice the standard error.

Uncontrolled fluctuations in feeding habits of the birds, due to shortening daylight hours while the experiments were in progress, and the small number of points at which determinations could be made, make it impossible to draw conclusions from minor trends in the curve. How-

ever, the three major trends previously mentioned are emphasized, namely: (1) the sharp decrease in glycogen content to a mere trace when food is withheld from the host, (2) a fluctuating daily cycle with a peak in the evening and a low point during the mid-morning and (3) a time lag between the beginning of feeding and the building up of a glycogen store. Comparison of curves in the glycogen cycle in the present work with those in the proglottid production cycle of Wetzel show marked similarity. From this it may be inferred that the latter cycle is dependent upon the former.

Exper. 5.—Although only entire worms which averaged 22.9 milligrams from starved chickens were used for analysis, the worms extracted

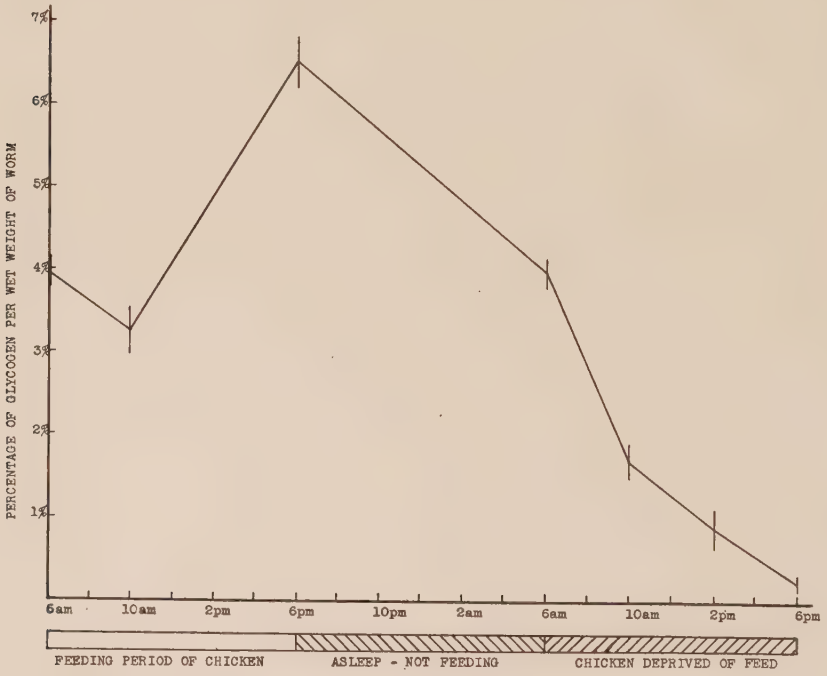

FIG. 1. Fluctuations in the glycogen level of tapeworms from chickens feeding normally, and during short periods of forced starvation.

were on the average a few milligrams lighter than those from unstarved chickens. Since this difference in weight was undoubtedly due largely to the loss of terminal proglottids in the worms from starved chickens, the question might be raised as to whether or not a glycogen gradient existed within each individual worm. Should the gravid proglottids which were lost prove to have a considerably higher glycogen level than that of the rest of the worm, the explanation given to the foregoing glycogen experiments would need to be reinterpreted.

Accordingly, an experiment was devised in which the 20 posterior segments from each worm were removed and separate analyses made on the posterior and the anterior portions of the worms. In small worms the 20 proglottids constituted about half the total weight of the worm, while in larger worms they made up a much smaller portion of the worm weight. Worms were obtained from chickens killed at 8 PM and 8 AM, so that further comparisons in the glycogen level between the evening and morning hours could be made.

The mean percentage of glycogen for the anterior ends of eight samples removed from three chickens at 8 PM was 6.54 while that of the posterior ends of these worms averaged 5.55 per cent. There was a difference of 0.99 per cent in the glycogen level in favor of the anterior ends, and the standard error being 0.295, the difference is highly significant. At the 8 AM hour the mean glycogen level for nine samples taken from four chickens was 5.01 per cent for the anterior ends while that of the posterior ends was 5.15 per cent. In this case the difference was not significant.

The results of these two groups of tests show that the glycogen level from the posterior proglottids was not significantly higher than that of the anterior portions. Although further experimentation would probably be necesssary to indicate the reason for the higher glycogen level in the anterior ends at the 8 PM hour, it appears improbable that a glycogen gradient could prejudice the conclusions drawn from the previous experiments.

As might be expected from the results of Experiment 3, the glycogen level at the 8 PM hour was higher than at the 8 AM hour, the 0.96 per cent difference in the means with a standard error of 0.250 is highly significant. Unfortunately the fowls, which were not accustomed to the operation of a new battery feeder, did not feed normally the day before the worms were removed, and the glycogen levels obtained in this experiment were not comparable with those indicated in Fig. 1.

PERCENTAGE COMPOSITION OF R. CESTICILLUS

Since reports of the percentage composition of other cestodes are available only in rather inaccessible sources, these data are presented in Table 4, where ready comparison can be made with the results obtained in the present experiments. It will be noted that the glycogen level for *R. cesticillus* with a mean of 6.51 per cent at the 6 PM hour is higher than the results obtained by other investigators with the exception of the 7.37 per cent recorded by Smorodinezew and Bebeschin (1935) for *T. saginata*.

FAT CONTENT AND STARVATION

Considerable interest has been shown in the fat metabolism of hel-

TABLE 4.—*Percentage composition of the strobilar phase of cestodes analyzed by various investigators expressed as the mean with the range given in parentheses*

Cestode	Investigator	Water %	Glycogen %	Ether soluble extract %	Total nitrogen %	Ash %
R. cesticillus	Reid	79.46 (75.5–83.1)	6.51 (3.84–8.51)	3.20 (1.35–4.62)	1.14 (1.04–1.41)	2.36 (1.86–3.95)
M. expansa	Wardle 1937a and 1937b	85–90	3.22* (1.55–3.96)*			1–2
" "	Weinland 1901	90.1 (89.5–92.2)	3.00† (1.5–4.7)†			
" "	v. Brand 1933	88.96 (86.79–91.51)	2.69† (0.99–5.35)	3.35 (1.91–4.56)	0.63 (0.56–0.76)	1.11 (1.02–1.20)
D. caninum	" " "	79.61				
T. marginata	" " "	76.44 (78.32–74.56)				
T. plicata	" " "	72.50		9.11		1.22
M. denticulata	" " "	91.42				
T. saginata	" " "	92.23				
" "	Smorodinezew, Bebeschin, and Pawlowa, 1933 1935, and 1936	87.82 (86.8–88.6)	7.37† (6.16–9.12)†	1.36 (0.29–3.17)	0.64 (0.54–0.72)	0.65 (0.52–0.85)
T. solium	"	91.29 (87.27–94.08)	2.17† (1.00–6.90)†	1.41 (0.85–2.77)	0.65 (0.47–0.93)	0.53 (0.29–1.03)
D. latum	"	90.50	1.90† (0.80–4.64)†	1.54	0.91	0.43

* Correction factor of 0.961.
† Probable correction factor 0.927.

minths because of the relatively high fat content, and the wide variability found in worms of the same species (Table 4). According to von Brand's view fats are byproducts of glycogen metabolism which probably remain as stored excretory material in the parenchyma of the worm. This conclusion was reached when relatively little fat was found in the excretory tubules of *M. expansa* as compared with large numbers of fat droplets found in the excretory sytem of the young fluke, *Fasciola hepatica* (Vogel and von Brand, 1933). Von Brand, however, found no significant change in fat content in nine experiments using in vitro starvation for six hours.

The results from ether extraction of 20 samples of *R. cesticillus* are summarized in Table 5. It will be noted that this species also has a

TABLE 5.—*Ether-extract and percentage of water in worms removed from chickens off feed for 16 to 20 hours and upon worms from unstarved chickens*

Treatment of worms	Total worms in sample	Water %	Ether-extract of wet weight %	Ether-extract of dry weight %
Unstarved Chickens killed between 2 PM and 6 PM	41	83.1	1.55	9.2
	14	77.5	2.78	12.2
	31	77.6	3.69	16.5
	73	82.6	4.48	25.8
	93	80.1	3.76	19.0
	11	76.3	4.62	19.4
	24	78.4	2.38	10.9
	11	75.5	4.05	16.6
	100	80.7	3.30	17.1
	26	82.8	1.35	7.9
	Means	79.46	3.20	15.5
Starved 16–20 hours Chickens killed between 2 PM and 6 PM	23	78.6	3.38	15.7
	48	83.4	3.24	19.6
	31	83.6	3.10	18.9
	38	83.5	3.08	18.4
	22	80.7	4.41	22.8
	39	85.7	1.62	13.0
	38	84.5	1.36	7.5
	25	81.2	1.90	9.9
	22	82.0	2.86	15.8
	45	86.5	2.00	15.0
	Means	82.97	2.70	15.5
	Difference in means	3.51*	0.50	0.0
	Standard error	1.226	0.466	...

* Statistically significant.

relatively high ether-soluble portion, the mean for the control samples being 3.20 per cent of the wet weight. In nine additional samples from worms killed in the morning hours, and therefore not included in the starvation table, the mean was 3.31 per cent with a range of 0.98–4.51 per cent.

Although the worms from starved chickens contained an average of 0.50 per cent less ether-soluble material than the control samples, the difference is not statistically significant. Of interest is the fact that the means are the same for the ether-soluble portion calculated on the basis of dry weight. Since a loss of approximately 30 per cent in the dry

weight of the worm would occur if all of the metabolic products of glycogen consumption during starvation were eliminated, a corresponding gain in the ether-soluble fraction might be expected if no actual change in its content had occurred. The present results might, therefore, be interpreted as indirect evidence for a decrease in the fat content during starvation.

The indications are that if an excess of fatty waste products was produced during starvation, due to the high glycogen consumption, these substances must have been excreted by the worm and did not accumulate in the parenchyma. On the other hand, the evidence is inconclusive for the opposite view that fats are a potential energy reserve, since the depletion is slight if a true loss does actually occur.

WATER CONTENT AND STARVATION

The water content of some thirty specimens was determined in connection with fat determinations (Table 5). It may be noted that the water content of the worms from starved chickens was 3.51 per cent higher than that from unstarved hosts. The standard error being 1.226, this difference is significant. Since there was a marked depletion in the glycogen content of the worm during starvation, a corresponding rise in water content might be expected. It should be noted, however, that starved hosts had water before them continually and therefore an increase in water content in the worms might be due to the increased percentage of water in their surroundings. The water content of this species, although not the lowest of the worms which have been analyzed, is lower than any of the other forms upon which complete determinations have been made. This is reflected in the higher percentage of glycogen, ash, and nitrogen reported for this species.

PROTEIN CONTENT AND STARVATION

Nitrogen determinations have been made on cestodes by several investigators to obtain an approximation of the protein present. The usual procedure is to multiply the total nitrogen by 6.25 to obtain the percentage of protein. Although no complete study of the protein content of cestodes has been made, Salisbury and Anderson (1939) in an analysis of the larval *Cysticercus fasciolaris* found that of 20 per cent of the total nitrogen which was dissolved in aqueous extracts of pulverized worm material, only seven per cent was precipitated by trichloroacetic acid and could therefore be regarded as protein. Likewise Eisenbrandt (1938) in a serological study of numerous helminths including the cestodes *Cittotaenia denticulata, Dipylidium caninum, Taenia pisiformis, Moniezia alba, M. expansa,* and *Thysanosoma actinioides* found the nonprotein fraction of the total nitrogen to be surprisingly high. Although

it would thus appear that the conversion factor will probably have to be revised when a more complete knowledge of the protein make-up of tapeworms has been obtained, the nitrogen content still remains the most convenient method of obtaining an estimate of protein content.

Five nitrogen determinations were made upon worms extracted from unstarved hosts killed at 6 PM and six determinations were made on worms removed from hosts which had been starved for 16 to 20 hours. Calculated on the basis of the wet worm weight, the worms from unstarved hosts averaged 1.14 per cent nitrogen while those from starved hosts averaged 1.37 per cent. The difference of the means is 0.24 per cent with a standard error of 0.093 which is statistically significant. Calculated on the basis of dry weight, worms from the starved hosts showed even a greater increase, nitrogen constituting 7.87 per cent of the weight as against 5.83 per cent for the control group. The difference in this case is 2.04 per cent with a standard error of 0.277 which is highly significant.

In the light of the glycogen experiments it seems probable that the increase in nitrogen is relative rather than actual. Since there is at least no loss of nitrogenous materials, and little if any loss of fat during the 20-hour starvation period, it appears that the starvation effect under consideration is due entirely to loss of glycogen. These findings are in keeping with the prevailing opinion that glycogen furnishes the main source of energy for cestodes.

ASH CONTENT

The total ash content of nine samples of worms taken from unstarved hosts was determined. The ash content averaged 2.36 per cent of the wet weight of the worm material with a range between 3.95 and 1.96 per cent. Calculated on the basis of the dry weight, the mean percentage of ash was 12.11 with a range between 9.82 and 17.07 per cent.

Although the strobilar ash contents of adult cestodes have not been analyzed, Salisbury and Anderson (1939) determined the following elements in the ash of the larval *Cysticercus fasciolaris* which constituted 16.3 per cent of the dry material: Na 6.99 per cent, K 3.66 per cent, Ca 29.5 per cent, Mg 20.14 per cent, S 0.74 per cent, P 7 per cent, with only traces of Cl and Fe.

THE EFFECTS OF CROWDING

A phenomenon which appears to be common to the larger species of cestodes that have thus far been studied experimentally is that crowding causes stunting in the size of the worms. Woodland (1924), Shorb (1933), and Hunninen (1935), have demonstrated this effect in the rat tapeworm *Hymenolepis fraterna*. Hunninen stated further that even in heavy infections a few worms may grow materially larger than others,

while some of the worms may remain immature for long periods. Chandler (1939) and Hager (1941) likewise presented evidence on the effects of crowding upon the size of *Hymenolepis diminuta.*

In the present studies the weights of some 1270 specimens of *R. cesticillus* from 44 chickens were available for comparison. Since weight is the most accurate measure of size and a much more dependable criterion in cestodes than the length or number of proglottids, these data are here presented. The results are summarized in Fig. 2. It will be noted that

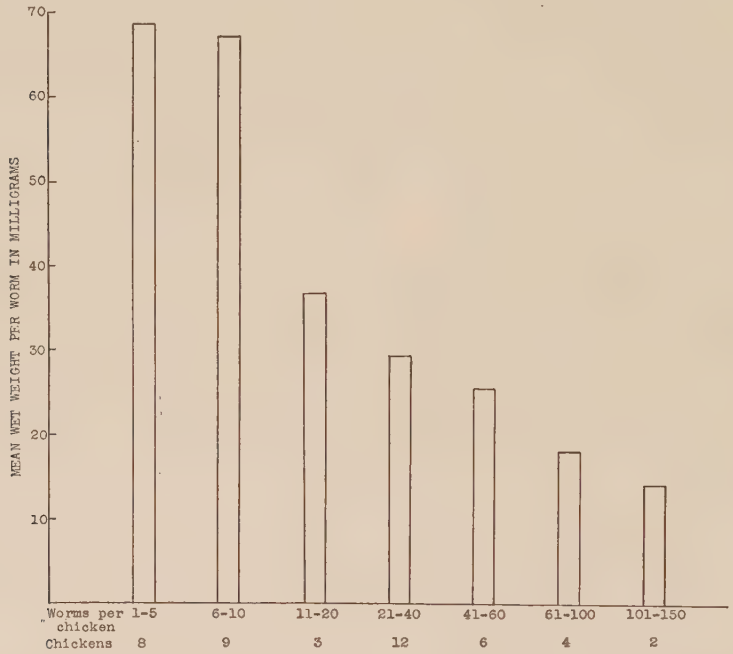

FIG. 2. The relative size of *R. cesticillus* in light and in heavy infections.

although little difference in size of worms is indicated between the one to five-worm group and the six to ten-worm group, a worm burden of over ten cestodes per bird results in a marked decrease in the size of the worms. Although the data available are insufficient to make numerical comparisons, it seems probable from observation that single worms reach greater sizes than do worms in two to ten-worm infections.

Four different causes have been suggested for this stunting effect in heavy infections. These are: (1) That a local immunity is developed in the host, (2) that insufficient food is available for all the tapeworms, (3) that excretory products of the worms inhibit growth, and (4) that an actual physical crowding takes place. There is some evidence against certain of these suggestions but no positive supporting evidence is available.

Chandler found that rats treated with an anthelmintic produced as large worms as similarly treated uninfected rats, and concluded that there is no evidence of a local immunity mechanism being involved in the dwarfing of tapeworms.

Even though it has been shown in the present studies that lack of sufficient carbohydrate in the gut of the chicken prevents the normal behavior of the tapeworms, the available evidence does not support the view that dwarfing in crowded infections is due to insufficient carbohydrate food. No significant difference in glycogen content of worms in heavy and in light infections could be found. Further support for the view that carbohydrate depletion is not responsible for dwarfing can be obtained indirectly by estimating the maximum amount of carbohydrate consumed by a heavy infection of worms. Judging from the data in Experiment 3, a large worm weighing 100 mg would scarcely be expected to consume more than 0.008 gm. of carbohydrate in a 24-hour period. Calculating from Henry's (1912) table, digestible carbohydrates would be present in the smallest amount of balanced ration consumed in a 24-hour period by any fowl under observation (16 gm), in excess of 8.5 gm. If 100 very large worms were present, not more than 10 per cent reduction in the sugar content of the gut would be expected. This would not appear to be sufficient to produce dwarfing. Hager (1941) showed experimentally that limiting the food of rats to one-half or one-third the normal intake had no effect on the reproductive rate of *H. diminuta,* reproduction likewise being inhibited by crowding.

WORM NUMBER AS DETERMINED BY PROGLOTTID COUNT

To compare the fowl hosts effectively, it became necessary to ascertain the approximate number of worms in a living chicken. In nematode studies such a procedure has been in use for many years, the worm number being determined by egg counts. Stoll (1936) by study of proglottid and onchosphere counts of the sheep cestode *Moniezia expansa* together with other biological data was able to determine single worm infections of this species. Levine (1938) was able to predict approximately the number of *Davainea proglottina* present in a fowl by proglottid count, each worm producing about one proglottid per day. Hager (1941) found no relationship between the onchosphere count and the number of *Hymenolepis diminuta* present in rats.

In estimating the number of *R. cesticillus* present in a fowl, two difficulties are encountered in making use of 24-hour proglottid counts. The most serious difficulty is related to the problem of crowding which has been discussed. From the results obtained in several hundred fecal examinations with various sizes of infections it may be concluded that in infections of one to ten worms, no crowding effect manifests itself in pro-

glottid production. In single worm infections or light infections, each worm produced an average of about nine proglottids per day. However, in heavy infections it was found that about four proglottids per day is a more usual number for each tapeworm. On this basis, low and high proglottid counts can be used with some accuracy. There is, however, an intermediate proglottid number by which determination cannot be made. The size of the proglottids may be of some value in making a determination under these conditions as gravid proglottids are larger in light than in heavy infections.

A second factor influencing the determination of worm numbers by proglottid counts is the duration of the infection. In the present studies occasional proglottids were recovered in the feces 11 days after the cysticercoids were fed. On the other hand, Jones is quoted by Stafseth (1935) as having noted the presence of the original terminal proglottid in certain worms a month after infection. A gradual increase in the number of proglottids recovered in the droppings of a fowl from the eleventh to about the thirty-fifth day after cysticercoids were fed was noted in the present work. It has been found necessary, therefore, to wait until about 30 days after infection to make a calculation by the proglottid count. A later gradual decline in the proglottid count is due to the loss of some worms, and to the more frequent periods of quiescence, in old infections.

The number of cysticercoids fed to a chicken may help in determining the number of tapeworms, but the percentage of infection is quite variable due to variations in hosts and in the viability of the cysticercoids. This knowledge, relative to cysticercoids ingested in combination with the proglottid count, may be helpful in estimation of the extent of infection. Estimation of worms present by recovering the original terminal proglottid from each worm has been found impractical. Although no simple formula can be applied to determine the number of worms in the host, the proglottid count combined with a knowledge of other biological factors makes possible a rough estimate of the minimum number of worms in a chicken.

DISCUSSION

The relatively high rate of glycogen consumption by the fowl tapeworm *R. cesticillus* revealed in the present study is somewhat surprising when the results are compared with in vitro studies which have been made on the sheep cestode *M. expansa.* The consumption of over 90 per cent of the total glycogen store in 20 hours of host starvation, as reported in Experiment 2, seems remarkable when compared with about 10 per cent of glycogen consumption during six hours with *M. expansa* as reported by von Brand (1933). It is questionable, however, as to how far the results obtained for the avian *R. cesticillus,* which does not usually

exceed 0.1 gram in weight, can be compared with the mammalian *M. expansa* which weighs several grams. The higher body temperature of the avian host would indicate a higher metabolic rate in *R. cesticillus*. Von Brand (1938) believed that the size of the parasite might determine the type of metabolism in a helminth. Nevertheless, in the light of the present studies, the importance of muscular contractions in overcoming the action of peristalsis, a factor mentioned by Wardle (1937a), might well be reemphasized.

Although the effects of variable secretion of digestive juices under starvation conditions could not be eliminated in the present experiments, the fact that glycogen loss occurred not only when the host was starved but also during the normal 24-hour feeding cycle, and the fact that immature cysticercoids do not develop until food is at hand (Reid, 1942), lends support to the view that the worms expelled after host starvation were actually starved.

Protozoologists have long recognized the effects of diet upon the parasite (Hegner, 1923, Becker, 1937, Becker and Morehouse, 1935, 1936, Becker and Derbyshire, 1937), but helminthologists have not given the matter much consideration. The facts established in the present studies, the evidence of a high carbohydrate requirement in cestodes presented by Levine (1938), and the experiments with vitamins by Hager (1941) constitute a growing body of evidence indicating that the food of cestodes is extremely important in maintaining normal conditions for proper growth and development. In studying the effects of deficient diets upon host resistance to *Hymenolepis fraterna,* Shorb (1933) noted a retardation in the growth of the parasite and some loss in worms which he attributed to the direct effect of the diet upon the parasite. Other studies on host resistance and deficient diets contain only incidental reference to the effects of these diets upon the parasite. In view of the present evidence it would appear that greater attention should be given to the effects of host diet upon the parasite, and that studies on diet and host resistance should be preceded with a knowledge of the nutritional requirements of the parasite involved.

SUMMARY

1. Twenty hours of host starvation is sufficient to reduce the glycogen content of the fowl cestode *Raillietina cesticillus* (Molin) to about one-eleventh of its normal level. This reduction in the glycogen store is believed to be responsible for the loss of strobilae in starvation because insufficient muscular energy is available to counteract the action of peristalsis.

2. A daily fluctuation occurred in the glycogen level of tapeworms in the gut of chickens under normal feeding habits from 7.14 per cent at 6 PM to 3.68 per cent at 6 AM. It is thought that this fluctuation,

which is dependent upon the presence of available food material in the host gut, explains the release of proglottids only at certain hours during the day. These findings afford proof of the importance of glycogen in the metabolism of cestodes.

3. The mean percentage composition of *R. cesticillus* removed from the fowl under normal feeding conditions at 6 PM was found to be 79.46 per cent water, 6.51 per cent glycogen, 3.20 per cent ether-soluble extract, 1.14 per cent nitrogen, and 2.36 per cent ash content. Compared with other work, the percentage of water is considerably lower than that in the other forms which have been completely analyzed, with correspondingly high glycogen, nitrogen, and ash values for this worm.

4. Crowding of *R. cesticillus* in heavy infections causes a dwarfing in the size the cestodes as measured by weight. The diminished carbohydrate level in the host intestine due to food consumed by the worm does not appear to be sufficient to produce this effect.

5. Proglottid counts supplemented with rather complete infection data, are of value in estimating the number of worms in a living fowl—information of value in anthelmintic and certain other experimental fields.

Bibliography

Ackert, J. E. and T. D. Beach 1933 Resistance of chickens to the nematode, *Ascaridia lineata,* affected by dietary supplements. Tr. Am. Micr. Soc. **52**: 51–58.

Ackert, J. E. and J. H. Whitlock 1941 Feeding habits of nemas (Parasitica). In "An Introduction to Nematology." J. R. Christie, Ed. Babylon, N. Y. Section II, Part 2: 350–355.

Ackert, J. E., J. H. Whitlock and A. E. Freeman, Jr. 1940 The food of the fowl nematode, *Ascaridia lineata* (Schneider). J. Parasitol. **26**: 17–32.

Alicata, Joseph E. 1939 Parasitology. Rep. Hawaii Agric. Exper. Sta. 66 pp.

———1940 Poultry parasites. Rep. Hawaii Agric. Exper. Sta. Rep., pp. 65–66.

Becker, Elery R. 1937 Dietary control in experimental coccidiosis. Science **86**: 403–404.

Becker, Elery R. and Neal F. Morehouse 1935 Effect of skim milk, lactose, vinegar and iodine on the quantitative character of a coccidian infection. Proc. Soc. Exper. Biol. and Med. **32**: 1030–1031.

——— and ——— 1936 The effect of diet on the coccidian infection of the rat. J. Parasitol. **22**: 60–67.

Becker, Elery R. and Russel C. Derbyshire 1937 Biological assay of feeding stuffs in a basal ration for coccidium-growth-promoting substance. II. Barley, rye, wheat bran, wheat flour middlings, soybean meal. Iowa State Coll. J. Sc. **12**: 211–215.

Brand, Th. von 1933 Untersuchungen über den Stoffbestand einiger Cestoden und den Stoffwechsel von *Moniezia expansa.* Z. Vergleich. Physiol. **18**: 562–596.

———1938 The nature of the metabolic activities of intestinal helminths in their natural habitat: aerobiosis or anaerobiosis? Biodynamica **41**: 1–13.

Burlingame, Paul L. and Asa C. Chandler 1941 Host-parasite relations of *Moniliformis dubius* (Acanthocephala) in albino rats, and the environmental nature of resistance to single and superimposed infections with this parasite. Am. J. Hyg. **33D**: 1–21.

CHANDLER, ASA C. 1939 The effects of number and age of worms on development of primary and secondary infections with *Hymenolepis diminuta* in rats, and an investigation into the true nature of "premunition" in tapeworm infections. Am. J. Hyg. **29**D: 105–114.

EISENBRANDT, LESLIE L. 1938 On the serological relationship of some helminths. Am. J. Hyg. **27**: 117–141.

FOLIN, OTTO AND HSIEN WU 1920 A simplified and improved method for determination of sugar. J. Biol. Chem. **41**: 367–374.

HAGER, ANNE 1941 Effects of dietary modification of host rats on the tapeworm *Hymenolepis diminuta*. Iowa State Coll. J. Sc. **15**: 127–153.

HEATLEY, NORMAN GEORGE 1935 The distribution of glycogen in the regions of the amphibian gastrula; with a method for the micro-determination of glycogen. Biochem. J. **29**: 2568–2572.

HEGNER, ROBERT W. 1923 The effects of changes in diet on the incidence, distribution and numbers of certain intestinal protozoa of rats. Am. J. Hyg. **3**: 180–200.

HENRY, W. A. 1912 Table III. Average digestible nutrients and fertilizing constituents in American feeding stuffs. In "Feeds and Feeding." 12th ed. Madison, Wis., pp. 582–589.

HUNNINEN, ARNE V. 1935 Studies on the life history and host-parasite relations of *Hymenolepis fraterna* (*H. nana,* var. *fraterna,* Stiles) in white mice. Am. J. Hyg. **22**: 414–443.

HSÜ, H. F. 1938 Studies on the food and the digestive system of certain parasites. II. On the food of *Schistosoma japonicum, Paragonimus ringeri, Dirofilaria immitis, Spirocerca sanguinolenta* and *Rhabdias* sp. Fan Mem. Inst. Biol., Zool. Ser. **8**: 347–366.

KERLY, MARGARET 1930 The solubility of glycogen. Biochem. J. **24**: 67–76.

LAPAGE, GEOFFREY 1937 Nematodes Parasitic in Animals. London. 172 pp.

LEVINE, P. P. 1938 Observations on the biology of the poultry cestode *Davainea proglottina* in the intestine of the host. J. Parasitol. **24**: 423–431.

MCCOY, O. R. 1935 The physiology of the helminth parasites. Physiol. Rev. **15**: 221–240.

MARKOV, G. S. 1939 Nutrition of tapeworms in artificial media. Compt. Rend. Acad. Sc. U.R.S.S. (Dok.). **25**: 93–96.

ORTNER-SCHÖNBACH, PAULINE 1913 Zur Morphologie des Glykogens bei Trematoden und Cestodens. Arch. Zellforsch. **11**: 412–449.

REID, W. M. 1940 Some effects of short starvation periods upon the fowl cestode *Raillietina cesticillus* (Molin). J. Parasitol. **26** Suppl: 16.

————1942 The removal of the fowl tapeworm *Raillietina cesticillus* by short periods of starvation. Poultry Sc. **21**: 220–229.

REID, W. M., J. E. ACKERT AND A. A. CASE 1938 Studies on the life history and biology of the fowl tapeworm *Raillietina cesticillus* (Molin). Tr. Am. Micr. Soc. **57**: 65–76.

SAHYUN, MELVILLE 1931 Determination of glycogen in tissues. J. Biol. Chem. **93**: 227–234.

SALISBURY, L. FRANK AND R. J. ANDERSON 1939 Concerning the chemical composition of *Cysticercus fasciolaris*. J. Biol. Chem. **129**: 505–517.

SHORB, DOYS ANDREW 1933 Host-parasite relations of *Hymenolepis fraterna* in the rat and the mouse. Am. J. Hyg. **18**: 74–113.

SMORODINZEW, I. A., K. W. BEBESCHIN AND P. S. PAWLOWA 1933 Beiträge zur Chemie der Helminthen. I Mitteilung: Die Chemische Zusammensetzung von *Taenia saginata*. Biochem. Z. **261**: 176–178.

SMORODINZEW, I. A. AND K. W. BEBESCHIN 1935 The content of glycogen in tapeworms (Cestoides). Compt. Rend. Acad. Sc. U.R.S.S. (Dok.) **3**: 413–414.

———— AND ———— 1936 Beiträge zur Chemie der Helminthen. III Mitteilung. Die Chemische zusammensetzung des *Taenia solium*. J. Biochem. (Japan) **23**: 19–20.

STAFSETH, H. J. 1935 On the control of tapeworm infestation in chickens with notes on the pathology of the intestines of the hosts. Mich. Agric. Exper. Sta. Tech. Bull. **148**: 1–46.

STOLL, NORMAN R. 1936 Tapeworm studies III. Sheep parasitized with one *Moniezia expansa* each. J. Parasitol. **22**: 161–179.

VOGEL, H. AND TH. VON BRAND 1933 Über das Verhalten des Fettes in den einzelnen Entwicklungsstadien von *Fasciola hepatica* und seine Beziehungen zum Exkretionssystem. Z. Parasitenk. **5**: 425–431.

WARDLE, R. A. 1935 Fish tapeworm. Biol. Board Canada, Ottawa. Bull. **45**: 1–25.

————1937a. The physiology of the sheep tapeworm, *Moniezia expansa* Blanchard. Canad. J. Res. **15D**: 117–126.

————1937b. The physiology of tapeworms. In "Manitoba Essays," 60th Anniv. Commemor. Univ. Manitoba, Winnipeg. Macmillan, pp. 338–364.

WEINLAND, ERNST 1901 Über den Glykogengehalt einiger parasitischer Würmer. Z. Biol. **41**: 69–74.

WETZEL, R. 1932 Zur Kenntnis des weniggliedrigen Hühnerbandwurmes *Davainea proglottina*. Arch. Wissensch. u. Prakt. Tierh. **65**: 595–625.

————1934 Untersuchungen über den Entwicklungskreis des Hühnerbandwurmes *Raillietina cesticillus* (Molin, 1858). Arch. Wissensch. u. Prakt. Tierh. **68**: 211–232.

WOODLAND, W. N. F. 1924 On the life cycle of *Hymenolepis fraterna* (*H. nana* var. *fraterna* Stiles) on the white mouse. Parasitology **16**: 69–83.

HYPODECTES CHAPINI N. SP. (ACARINA) FROM THE RED-SHAFTED FLICKER

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A red-shafted flicker (*Colaptes cafer collaris*) captured February 16, 1940, at Davis, California, and held in captivity until April 4, showed, on autopsy, 202 specimens of an immature acarian loosely encysted in the connective tissue surrounding the trachea, and, to a lesser extent, the esophagus. The greatest concentration was near the point of entrance of the trachea into the buccal cavity, but the area of infection extended from the lungs to the tongue. Another flicker, brought directly from the field on April 5, showed essentially the same picture, although the infection was not so heavy.

No movement was visible within the cysts as far as could be determined, and there was no apparent pathology in the host tissue at the site of the infection. When the parasites were placed in water the only definite movements were the slow waving of the legs, although the abdominal constriction appeared to become more pronounced in some individuals after a few minutes. No locomotion was observed in water on a tissue or glass surface.

These mites have been identified as a new species of the genus *Hypodectes* Filippi (1861). Giebel (1861) published descriptions of certain mites that had been collected by Nitzsch from the internal tissues of birds, erecting for them the genus *Hypoderas*. Later (1871) Giebel pointed out that Filippi's genus *Hypodectes* and the genus *Hypoderas* were synonymous but maintained that the generic name *Hypoderas* should be retained because it was an older genus (*Hypoderas* had been an unpublished manuscript name of Nitzsch) and because Filippi had named only 1 species in his genus (actually 5 species were named) while *Hypoderas* was supported by 12 species. Giebel also objected to Filippi's procedure of naming *Hypodectes nycticoracis* after the host, since *Ardea nycticorax* shelters two species of the genus.

A comparison of the dates of publication of the original descriptions of these two genera shows that Filippi published in June, 1861, while Giebel's paper appeared in October–November of the same year. The present authors therefore consider that the correct generic name should be *Hypodectes* and that Giebel's reasons for retaining the name *Hypoderas* were inadequate.

Received for publication, November 14, 1941.
* The writers are gratefully indebted to Dr. M. A. Stewart, Dr. R. L. Usinger, and Dr. T. I. Storer for suggestions and criticisms.

Robin and Mégnin (1877) published observations on the life history of *Hypodectes columbae* Slosarski found in pigeons and showed that it was a hypopial nymph of *Pterolichus falciger,* a feather mite, now called *Falculifer rostratus* Buchholz. According to these authors, the normal nymph molts into the hypopial stage during adverse environmental conditions, as during molting of the bird, etc. The hypopial forms migrate internally through feather follicles or respiratory organs of the bird host and come to lie in subcutaneous or tracheal tissue, there to live and grow until such time as external conditions become normal when they return to the outside and take on their original form. This deviation of the normal life cycle is supposed by these authors to be a means of saving the colony from destruction.

In view of the findings of Robin and Mégnin (*loc. cit.*) the genus *Hypodectes* probably has no true taxonomic standing, but like the helminth genera *Cercaria, Cysticercus, Microfilaria,* etc., it is used to designate immature forms for which the adult stages may be unknown.

Since the life history of the present form has not been studied it seems advisable to establish a new species for its reception until such time as the taxonomic position of the adult stage has been definitely determined.

The authors take great pleasure in naming this species after Dr. E. A. Chapin who has been kind enough to refer us to information on the affinities of this species.

Hypodectes chapini n. sp.

(Fig. 1)

General appearance white and smooth with brownish sclerotized areas; measurements 0.54 to 1.15 mm long by 0.22 to 0.42 mm wide (through middle of cephalothorax); anterior end abruptly truncate; end of abdomen evenly rounded. Cephalothorax convex dorsally and flattened ventrally; triangular area with sclerotized margins present on anteroventral surface; no trace of anterior body opening, sensory papillae, or penetrating apparatus. Mouth-parts absent. Legs, eight in number, two pairs anterolaterally and two pairs posterolaterally on ventral side of cephalothorax. Four distinct segments plus a terminal appendage on all legs; basal segment largest; terminal appendage with one long and one or two short hairs. Legs articulate to sclerotized supports on body wall. Small shelf-like projection of body wall dorsal to each posterior group of legs. Only body opening detected, a median slit with two lateral lips connected by muscle fibers to body wall, located in medio-ventral line between fourth pair of legs. Abdominal constriction, between cephalothorax and abdomen, very marked in some specimens so that abdomen appears heart-shaped; constriction less distinct in others. Abdominal constriction traversed ventrally by one large and in some specimens one or two pairs of small sclerotized areas which appear thickened internally; sides of constriction with small indistinct sclerotized area laterally. Posterior end of abdomen with sclerotized cap traversing abdomen ventrally, also dorsally to abdominal constriction; abdomen, like cephalothorax, rather flattened ventrally and convex dorsally. Degree of sclerotization variable, some individuals with little trace of terminal sclerotized cap or triangular area and less sclerotization of leg supports; no constant correlation between body size and degree of sclerotization.

Internal structures (observed in whole mounts and by micro-dissection) are muscle fibers on body wall, and many large cells containing spherules and granules

of various sizes; large cells appear to contain much fatty material. Digestive tract lacking. Muscle fibers extend from body wall to lips of body opening and to legs. Cuticle of body wall smooth externally, but covered on inner surface with many small tubercles and ridges, especially in areas of heavy sclerotization.

Host.—*Colaptes cafer collaris* Vigors (Red-shafted flicker).

Location.—Connective tissue cysts on trachea and oesophagus.

Locality.—Davis, California.

Type.—Type and 4 paratypes in U.S.N.M. No. 1383, remaining paratypes in senior author's collection.

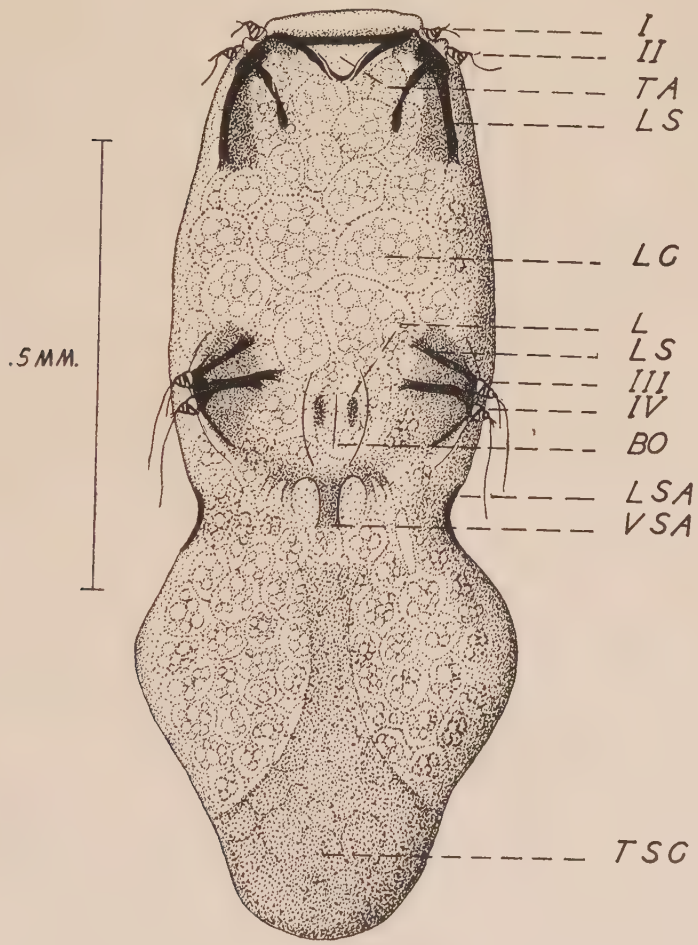

FIG. 1. *Hypodectes chapini*

Drawing by camera lucida—ventral view.

I, II, III, IV—legs

BO—body opening
L—lip of body opening
LC—large cell
LS—leg support
LSA—lateral sclerotized area
TA—triangular area
TSC—terminal sclerotized cap
VSA—ventral sclerotized area

Hypodectes chapini may be distinguished from the other species of the genus by the abdominal constriction behind the last pair of legs, the lack of body setae except on the legs, and the sclerotization pattern of the body.

The genus *Hypodectes* has been reported from the United States in domestic pigeons by Garman (1884), Kellicott (1892), and Ward (1894), and from the little blue heron (*Florida caerulea*) by Leidy (1890). Beebe (1902) reported mites (apparently of the genus *Hypodectes*) from subcutaneous tissue on each side of the keel of the sternum in the white ibis (*Guara alba*) in the New York Zoological Park. He also noted similar forms in the little blue heron (*Ardea caerulea*), the great-crowned pigeon (*Goura coronata*), the Nicobar pigeon (*Calaenus nicobarica*), and the roseate spoonbill (*Ajaja ajaja*). This author ascribes mortality among these birds to the presence of the mites. Ward (1902) doubts that the mites were the cause of mortality in these instances. Several of the bird hosts noted by Beebe (1902) were already known to harbor various species of *Hypodectes,* a fact of which Beebe was apparently unaware.

Dr. E. A. Chapin states (personal correspondence) that he has recovered specimens of *Hypoderas* (= *Hypodectes*) several times from postmortem examination of wild birds.

Hypodectes chapini n. sp. is evidently the first member of this genus that has been recorded for *Colaptes cafer collaris* and the second species to be specifically identified in North American birds, the first being *H. columbae* mentioned above.

Bibliography

Banks, N. 1915 The Acarina or mites. U. S. Dept. Agric. Rep. No. 108, pp. 1–153.

Beebe, C. W. 1902 Preliminary observations on a subdermal mite occurring among the birds in the New York Zoological Park. Science **15**: 754–755.

De Filippi, Filippo 1861 Note zoologiche di Filippo de Filippi. I. *Hypodectes* nuovo genere di acaridi proprio degli uccelli. Arch. Zool., Anat. e. Fisiol., **1**: 52–60.

Garman, H. 1884 *Pterolichus falciger,* Mégnin, observed in the United States. Am. Naturalist **18**: 430–431.

Giebel, C. 1861 Die Milbenarten der Gattung *Hypoderas* Nitzsch. Z. Ges. Naturw. **18**: 438–444.

———1871 Ueber einige Milben. Idem **27**: 29–32.

Kellicott, D. S. 1892 *Hypoderas columbae.* A note. Insect Life **5**: 77–78.

Leidy, J. 1890 *Hypoderas* in the little blue heron (*Florida caerulea*). Proc. Acad. Nat. Sc., Phila. 1890, p. 63.

Robin, Ch. and P. Mégnin 1877 Mémoire sur les sarcoptides plumicoles. X. Description particulière des genres et des espèces de sarcoptides plumicoles. J. Anat. et Phys. **13**: 391–429.

Ward, H. B. 1894 On the pigeon mite *Hypodectes* Filippi. Pysche **7**: 95–100.

———1902 The subdermal mite occurring among birds. Science **15**: 911.

RESEARCH NOTES

A MODIFICATION OF ZINC SULFATE CENTRIFUGAL FLOTATION METHOD FOR RECOVERY OF HELMINTH OVA IN FORMALINIZED FECES

The zinc sulfate centrifugal flotation technic (Faust et al, 1938, Am. J. Trop. Med. 17: 79–84) was substituted for the Willis brine levitation technic in order to detect both protozoan cysts and helminth ova. This was done at a time (Sept., 1940) when formalinized fecal specimens were being examined in this laboratory. In subsequent fecal examinations zinc sulfate CF was found to yield a low incidence of hookworm in specimens coming from regions of this state where a high hookworm incidence was known to be present.

In addition, zinc sulfate CF as originally described was found to be excessively time consuming in this laboratory where fifty to seventy-five thousand fecal specimens are examined annually.

Different modifications of the zinc sulfate CF method were tested and the results compared with those obtained by the Willis' brine levitation method. Every formalinized fecal specimen was thoroughly comminuted and divided into two equal portions before being submitted to any diagnostic procedure.

What was considered to be a satisfactory modification of the zinc sulfate CF method was found, and this paper presents the results obtained with the original method and the modified method. In each case the results were compared with those obtained by Willis' brine levitation.

The following is a brief description of the methods used in this study:

(1) The zinc sulfate CF using zinc sulfate solution of specific gravity 1.180; the preparations of feces were strained through cheesecloth, washed two times, and the surface layer material recovered by the superimposed coverslip method. This is the same as that described by Faust et al (1938).

(2) Willis' brine levitation. The emulsified specimens were poured into small metal boxes, the latter filled with brine specific gravity 1.200 and a large glass fecal slide superimposed over each box. After five or ten minutes these slides were examined.

(3) A modified zinc sulfate CF similar to No. 1, except that a zinc sulfate solution specific gravity 1.200 was used and cheesecloth straining and the second washing were omitted. The surface layer material was removed by the superimposed coverglass method.

Centrifugation time was the same in both centrifugal flotation procedures.

A preliminary series of 100 formalinized specimens was examined by methods (1) and (2). These were thoroughly comminuted and divided into two equal portions. All had been in contact with 3–4 per cent formalin for about two weeks, and were from a region of high hookworm incidence. Method (1) revealed 10 (10 per cent) positives for hookworm and method (2), 60 (60 per cent) positives. Method (1) did not reveal any positives not found by method (2).

A second group of 1,222 formalinized fecal specimens obtained from a wide variety of places in Florida was examined by methods (2) and (3). The results of this series are presented in the accompanying table.

TABLE 1.—*Incidence of helminth infections revealed by methods (2) and (3) in 1,222 fecal examinations*

Eggs of	NaCl. lev. No. (2)		$ZnSO_4$ CF No. (3)		Total incidence
	Number	Per cent	Number	Per cent	
Hookworm	407	33.3%	403	32.1%	470
Ascaris	25	2.0%	31	2.4%	31
Whipworm	10	0.8%	7	0.6%	12
Pinworm	9	0.7%	9	0.7%	15
Tapeworm (*H. nana*)	0	0.0%	2	0.1%	2
Total helminths	451	36.9%	452	37.0%	530

In the first series of 100 fecal examinations no positive specimens were found by zinc sulfate CF that were not found by Willis' brine levitation. Thus 60 represents the total incidence for hookworm, the brine revealing 50 more than the zinc sulfate CF.

In the second series of 1,222 this was not the case. Zinc sulfate revealed 340 (a) cases of hookworm that were also found positive by the Willis' brine levitation method. The Willis' brine levitation revealed an additional 67 (b) cases missed by zinc sulfate CF. Thus Willis' brine levitation revealed (a) plus (b) (340 plus 67), or 407 positives. On the other hand, zinc sulfate CF revealed hookworm in an additional 63 (c) cases missed by brine. Zinc sulfate revealed (a) plus (c) (340 plus 63), or 403 positives. Thus, (a) plus (b) plus (c) (340 plus 67 plus 63) equals 470, or the total number of specimens positive for hookworm in the 1,222 specimens examined by these two methods.

The increased efficiency as a result of the modification of the zinc sulfate CF is obvious. In the first 100 specimens the Willis brine method found 50 per cent more hookworm than the zinc sulfate CF, while in the latter 1,222 specimens, the brine recovered only 1.2 per cent more hookworm than zinc sulfate CF. Furthermore, the time required to perform method (2) was less than half that required for method (1).—William A. Summers, *Senior Parasitologist, Bureau of Laboratories, Florida State Board of Health, Jacksonville, Florida.*

THE PERIODICITY OF OÖCYST DISCHARGE IN COCCIDIAL INFECTION OF CHICKENS

The periodicity of oöcyst discharge in sparrows and pigeons affected with coccidiosis has been demonstrated by Boughton (1932, J. Parasitol. **19**: 177; 1934, Ibid. **20**: 329; 1937, Ibid. **23**: 291–293). The experiments described herein were performed to determine whether or not a similar phenomenon characterized the development of coccidial oöcysts of the chicken.

Coccidia-free White Leghorn males, seven weeks old, were used. Five groups of nine to ten birds each were dosed into the crop with suspensions of sporulated oöcysts of *Eimeria praecox, E. mitis, E. maxima, E. hagani* and *E. necatrix,* respectively, in the month of July. The birds in each group received the same number of oöcysts, varying from 800 of *E. maxima* to 8,900 of *E. necatrix.* By mistake, the dose of *E. hagani* was much larger (300,000). The doses were kept small purposely to avoid producing severe infections which would alter the feed intake, character of the feces, etc. *E. hagani,* a non-pathogenic species, had no effect on the birds despite the size of the dose. Each group was dosed at 9 AM at a date so chosen that the first discharge of oöcysts resulting from the infection occurred on the same day in every group. In this way there was assured a uniformity in the environmental influences that might have had an effect on the oöcyst discharge of the different species. Sterilized holding batteries with wire screen floors and removable dropping pans housed the five groups. An adequate all mash ration was fed. Feces of each group were collected and weighed at 9 AM, 3 PM and 9 PM. Oöcyst counts were made according to the method already described (Levine, 1939, Cornell Vet. **29**: 309–320). The total number of oöcysts discharged and the percentages of the total daily oöcyst and fecal discharges for each of the three periods of the 24 hours were computed and graphed. *E. acervulina* was not available at the time this study was made. *E. tenella* was not used since infection with this species, in many instances, causes the formation of a caseous plug in the cecal lumen, thereby preventing the discharge of oöcysts from that organ.

The results are indicated in the series of graphs in Fig. 1. The amount of feces collected during each of the three periods of the day was about the same. There was a tendency for a slight increase during the 9 AM to 3 PM interval. The discharge of oöcysts, however, fluctuated in a periodic manner, although the steepness of the curves for each of the species was not the same. The most marked periodic discharge was shown by *E. hagani.* During the six hours from 3 PM to 9 PM, 87% to 91% of the total daily output was eliminated. An insignificant num-

ber of oöcysts were eliminated during the rest of the day. Two other trials with *E. hagani* on different occasions yielded similar results. None of the other species discharged so many oöcysts in such a short time. The number of oöcysts of *E. praecox* reached a peak during the same time interval but the discharge during the

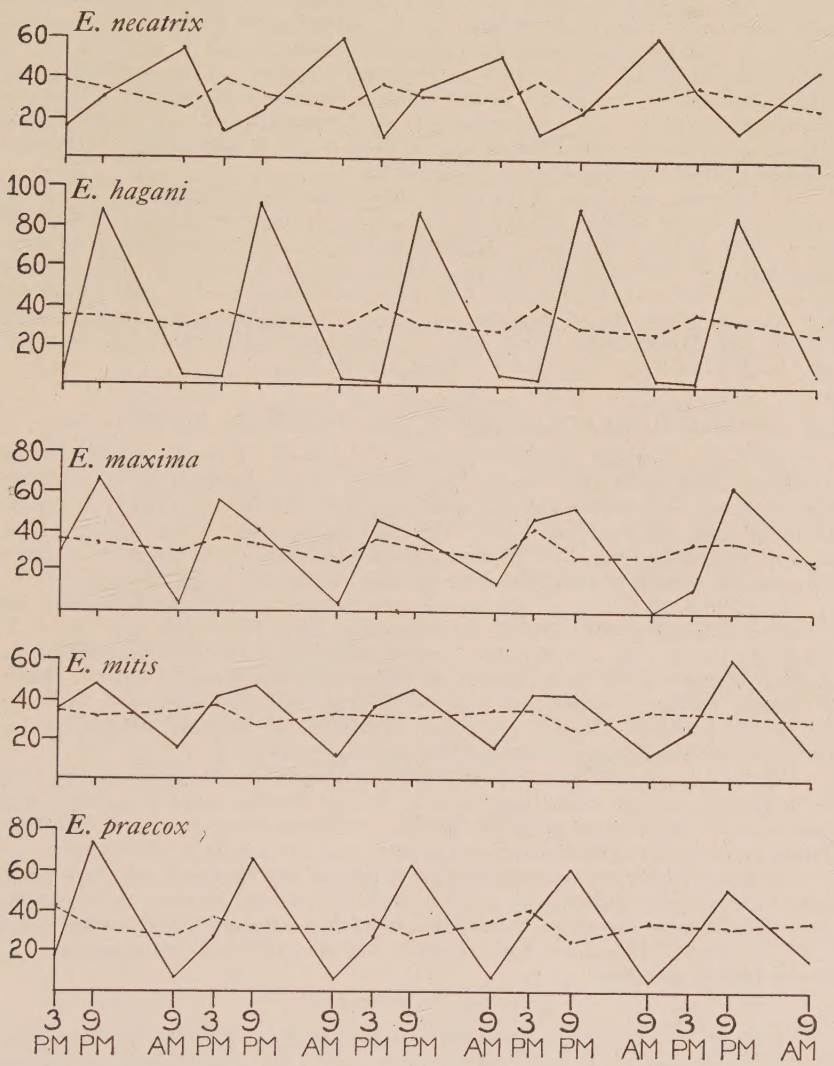

FIG. 1. Periodic fluctuations in the percentages of oöcysts (solid line) and feces (broken line) discharged by experimentally infected birds during the periods between 9 AM–3 PM–9 PM.

rest of the day accounted for from 26% to 43% of the total. The same was true for *E. mitis* except that the curve was flattened still more, since from 37% to 55% of the oöcysts were discharged during 9 PM to 3 PM the following day. The results with *E. maxima* were somewhat erratic since the peak of the curve was not reached during the same period each day. On three days, the greatest number was discharged during the 3 PM–9 PM interval while on two days the peak was reached

between 9 AM and 3 PM. With the exception of the last day, the discharge of oöcysts of *E. maxima* was smallest during the 9 PM–9 AM period. *E. necatrix* differed from all the other species in that the greatest oöcyst discharge occurred during the night and early morning, between 9 PM and 9 AM. From 47% to 62% of the oöcysts were eliminated during this time. One cannot conclude, however, from examination of the graph, that the formation of oöcysts of *E. necatrix* occurs periodically even though the apparent discharge makes it appear so. The oöcysts of this species are produced in two cecal pouches. These organs not only empty themselves independently of one another, but also independently of the rest of the intestinal tract. Consequently, until some way is found to collect the cecal discharges separately from those of the intestine, it will be impossible to determine whether there is true periodicity in this species.

Undoubtedly, the shape of the curves might have been different had this study been made during another time of the year when the number of hours of daylight are smaller, or had other time intervals been chosen. However, since all of these trials were run simultaneously, the basis for comparison is identical. The periodicity of *E. hagani* is so characteristic, that it may be used as another criterion for the identification of this species in addition to those described previously (Levine, 1938, Cornell Vet. **28**: 263–266).—P. P. Levine, *Department of Pathology and Bacteriology, New York State Veterinary College, Ithaca, N. Y.*

PARASITOLOGICAL SURVEY OF LOUISIANA MUSKRATS

This paper contains a general report of the parasites of the Louisiana muskrat (*Ondatra zibethica rivalica* Bangs), with notes on the incidence of some of the species found. All of the muskrats were trapped in 1939–40 and 1940–41 in several localities along the Louisiana coast. Principal among these is the Willow Bayou area of the Sabine National Wildlife Refuge in Cameron Parish, the Vermilion Bay area in Vermilion Parish, and the Michaud marsh in Orleans Parish.

In addition to Ernest C. Martin for collaboration on parts of this study, the writer is indebted to Dr. Carl E. Venard, Ohio State University, and Dr. J. E. Shillinger, U. S. Fish and Wildlife Service, respectively for identifications of the pentastome and most of the helminths; to Florence Brooks Kreeger and Dr. Harley N. Gould, Tulane University, for many of the carcasses examined; and, to Harry E. Adams, Julian A. Howard and others of the Sabine National Wildlife Refuge personnel for cooperation during the course of the work.

Of the total of 1,780 muskrats examined in the past two trapping seasons, only 53 were more or less completely searched for all parasites; the remainder were examined for only several particular species. All autopsies were made in the laboratory from freshly-skinned carcasses as they were secured from the trappers.

Because of their semi-aquatic habitat, natatorial predilections, and often heavy concentrations one would expect muskrats to be subject to infections by large numbers of helminths, especially trematodes, and other parasites. Svihla and Svihla (1931, J. Mamm. **12**: 12–18) have reported that muskrats of Louisiana were practically free of parasites.

EXTERNAL PARASITES

Probably because of the condition of the marshes at the time of the studies, during which rainfall was good and the water level at an optimum depth in the runways, very few external parasites were observed.

Mites—Tetragonyssus spiniger Ewing & Stover were most abundant in the nests of the young muskrats. At Willow Bayou they were present on about one-fourth of the muskrats, but there were seldom more than one or two to the animal. Trappers in all parts of the state report that in drought years the muskrats become very heavily infested with mites and that at such times their own clothes must be changed frequently to get rid of the "vermin"; these mites, however, seem to die soon after leaving the fur of the host. Svihla and Svihla (1931) noted mites on muskrats at Avoca Island near Morgan City, St. Mary Parish, La.

Diptera.—Several larvae of the flesh-fly, *Sarcophaga* sp., were found in the fur of two muskrats from Willow Bayou.

INTERNAL PARASITES

Protozoa.—Two species were found in the intestines of some muskrats. *Giardia,* apparently the recently described *G. ondatrae* (Travis, 1939, J. Parasitol. **25**: 11–17), was of common occurrence in all of the intestinal smears examined, and small flagellates, probably *Trichomonas* sp., were also found in several duodenal smears.

Nematodes.—Capillaria hepatica (Bancroft) occurred in 10 percent of Michaud muskrats, nearly 50 per cent of Vermilion Bay 'rats, and was completely absent at Willow Bayou. *C. hepatica* has been recorded from muskrats also by Price (1931, J. Parasitol. **18**: 51) and Warwick (1936, Parasitology **28**: 395–401).

Rictularia sp. and *Physaloptera* sp. were found in small numbers in about one-fourth of both the Michaud and Willow Bayou animals. Chandler (1941, J. Parasitol. **27**: 175–181) has just described *Rictularia ondatrae* from muskrats in southeastern Texas.

Longistriata adunca was found in one muskrat from Willow Bayou.

Tapeworms.—Only two were encountered, both from Willow Bayou, and have been identified as *Hymenolepis evaginata.*

Trematodes.—In spite of the tremendous number of flukes encountered in the small intestine of the Michaud muskrats, apparently only three species were present. The most common by far was the small monostome *Nudacotyle novicia* Barker. In some muskrats they covered the walls of the intestine, very large numbers being recovered in zinc sulfate flotations. This species, however, was of rare occurrence in the Willow Bayou area, nor did Chandler (1941) find it abundant in east Texas.

Echinochasmus schwartzi Price was of high incidence at Willow Bayou, but rare at Michaud. This species was the most abundant of those found by Chandler (1941) in east Texas.

A few specimens of *Paramonostomum pseudalveatum* Price, were found in a single muskrat from Willow Bayou.

Pentastomes.—A nymphal pentastome of the family Porocephalidae, *Porocephalus crotali* (Humboldt), infecting mainly the liver and lungs occurred in 9% of the muskrats from the whole state. Infection by this parasite has been studied in detail and is reported elsewhere (Penn and Martin, 1941, J. Wildlife Management **5**: 13–14; and Penn, 1942, J. Parasitol. In press).

SUMMARY

Thirteen species of parasites are recorded from Louisiana muskrats. These include one mite, one flesh-fly larva, two flagellated protozoa, four nematodes, three trematodes, one tapeworm, and one nymphal pentastome.—GEORGE H. PENN, JR., *Wildlife and Fisheries Division, Louisiana Department of Conservation, New Orleans.*

COCHLOSOMA ROSTRATUM FROM THE TURKEY

In 1923 Kotlan (Centr. Bakt. I Abt. Orig. **90**: 24–28) described as a new genus, *Cochlosoma,* a flagellate found in the cecum of European domestic ducks. He named this species *Cochlosoma anatis.* He found it in ducks which showed inflammation of the intestinal tract.

In 1934 Kimura (Tr. Am. Micr. Soc. **53**: 102–115) found a representative of the same genus in Muscovy and white Pekin ducks in California. Certain morphological characteristics, principally the shape of the sucker, convinced him that it was a new species, which he named *Cochlosoma rostratum.* He found it to be widespread in occurrence in ducks in this state, although more abundant on ranches than in market birds. *Cochlosoma rostratum* measures 6–10 × 3.9–6.7 microns. The most characteristic organelle is the ventral sucker which is half the length of the body and in fresh preparations reminds one of *Giardia,* although the curvature and

depth of the sucker is more pronounced. "The characteristic movement is constant circling in a very small radius with a spiral twisting of the body." This is in contrast to the jerky movement of *Trichomonas,* or to the rapid darting motion of *Hexamita.*

In our studies of infectious catarrhal enteritis (hexamitiasis) of turkey poults (1938, Cornell Vet. **28**: 281–293) we have observed a flagellate which is indistinguishable morphologically from *C. rostratum.* In young poults we have found it throughout the intestinal tract, and in adults in the region of the cecal tonsil. Kimura found it usually only in the large intestine and occasionally in the lower ileum of ducks. Unlike Kotlan he felt that it was of no pathogenic significance. Since the age of the ducks Kimura examined is not clear, it is possible that in ducklings the organism may be found throughout the intestine. In turkey poults we have always found it associated with *Hexamita* or in combinations of *Hexamita* and *Salmonella.*

The true significance of this parasite in turkey poults or ducklings has not been determined.—E. McNeil and W. R. Hinshaw, *University of California, Division of Veterinary Science, Davis, California.*

A SEVEN-YEAR-OLD INFECTION OF *CRYPTOCOTYLE LINGUA* CREPLIN IN THE WINKLE *LITTORINA LITTOREA* L.

In 1935 I collected several hundred specimens of *Littorina littorea* L. One of the largest of these molluscs, measuring 33 mm in length, was infected with the trematode *Cryptocotyle lingua* Creplin. It has been kept by me since that date in filtered sea water, and is incidentally the only one of my experimental animals to survive the Plymouth "blitz." In a letter (Nature, 1940, **146**: 367) I recorded that approximately 5,500,000 cercariae had emerged during five years, and in August 1938 the mean daily count was still 830. Curiously this figure has now risen to approximately 1,600 cercariae per 24 hours.

As filtered sea water has been used throughout the experiment, any possibility of re-infection is precluded. I think, therefore, it is worth while drawing attention to the fact that a single infection has survived nearly seven years and is still producing thousands of cercariae. This is in itself no evidence against the current theory of germinal lineage, to which many helminthologists subscribe, but the implications are interesting, and it certainly raises several points relating to the phenomenon which require investigation.—Miriam Rothschild, *Ashton Wold, Peterborough, England.*